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Influence of primary and secondary plant metabolites on the migration and feeding behavior of *Cacopsylla pruni*, the vector of European Stone Fruit Yellows

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Influence of primary and secondary plant metabolites on the migration and feeding behavior of *Cacopsylla pruni*, the vector of European Stone Fruit Yellows

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„Die Neugier steht immer an erster Stelle eines Problems, das gelöst werden will."

– Galileo Galilei –

Contents

Abstract

The plum psyllid *Cacopsylla pruni* is a univoltine herbivore, specialized on *Prunus* and coniferous tree species. During their lifetime, plum psyllids are alternating twice between their deciduous and evergreen hosts. For reproduction, *C. pruni* adults migrate to stone fruit orchards in spring, where they lay their eggs exclusively on several *Prunus* species. Adults of the old generation die after mating and oviposition. Young adults emerge from egg to adults during April, May and June. After several days the young adults (called emigrants) emigrate to conifers in higher regions until they remigrate (remigrants) to *Prunus* orchards in the next spring.

Plum psyllids transmit the Phytoplasma '*Candidatus* Phytoplasma prunorum' and are therefore of significant importance for fruit growers. In host plants, the wall-less bacterium is restricted to the phloem and causes the European Stone Fruit Yellows (ESFY). Psyllids acquire the bacteria during feeding on the phloem of infected *Prunus* trees. After multiplication of the phytoplasma inside the vector, plum psyllids transmit the disease to non-infected *Prunus* trees by salivary excretion during feeding. *C. pruni* is distributed all over Europe and bordering areas. ESFY is one of the most serious plant diseases in European fruit production, causing severe plant damage leading to a poor harvest and high economic losses. Peaches (*Prunus persica*), apricots (*Prunus armeniaca*) and Japanese plums (*Prunus salicina*) are worst affected by typical symptoms, such as reduced dormancy, chlorotic leaf roll and premature ripening of the fruits. Trees of these species suffer severely from the infections, decline and finally die. Commonly indigenous *Prunus* species, such as blackthorn (*Prunus spinosa*) und wild plums (*Prunus cerasifera, Prunus insititia*) show more tolerance towards ESFY infections. Likewise, most of the cultivated varieties of European plums (*Prunus domestica*) do not develop severe symptoms.

To date no effective control agents or cures for phytoplasma diseases are available. The control of vector insects is an alternative strategy. Psyllid behavior could be manipulated with infochemicals and prevent *C. pruni* from feeding and oviposition on stone fruit crops and thus reduce the number of new infections. In this thesis I investigated the impact of plant-borne volatiles and phloem chemistry on the behavior of *C. pruni* as yet barely anything is known about the biology and chemical ecology of plum psyllids.

The field monitoring presented in this thesis proved a preference of *C. pruni* for some *Prunus* species over others. *P. insititia* was identified as a favored host of *C. pruni*, in contrast very low numbers of plum psyllids were detected on *P. insititia* trees, which was therefore categorized as a non-preferred host for *C. pruni*. In the studies of this thesis, I compared the volatile organic compounds and the phloem sap composition of these two *Prunus* species and Conifers, to identify signals that were important for host plant preference of *C. pruni*. I demonstrated the detection of

volatile compounds characteristic for *Prunus* trees as well as characteristic coniferous host volatiles of female plum psyllid antenna by electroantennography. Olfactometer tests revealed that this preference is not only based on olfactory cues. Additionally, gustatory cues seem to play a major role in host acceptance and preference. *C. pruni* nymphs showed greater development success on preferred wild plum species (*P. insititia*) compared to nymphs on non-preferred peach trees (*P. persica*). Next to effects on psyllid development, I detected differences in the phloem composition of both plant species.

My research on the feeding behavior of plum psyllids on coniferous diets revealed that although *C. pruni* nymphs showed feeding on conifer needles, they are not able to develop on conifers. In contrast, adult plum psyllids survived longer on spruce (*Picea abies*) and silver fir (*Abies alba*) than without food supply. I concluded that adult *C. pruni* need evergreen tree species as resource of water and nutrition during overwintering.

Furthermore, I investigated the impact of '*Ca.* P. prunorum' infections of *Prunus* trees on the interaction between vector insects and their host plants. For this purpose, I recorded the feeding behaviour of *C. pruni* nymphs on infected and non-infected *P. insititia* and *P. persica* trees by electropenetrography. Interestingly, the average duration each nymph spend with the ingestion of xylem was shorter on infected than on non-infected *Prunus* trees. I found no influence on the average duration of phloem phases per nymph due to the infection status of both *Prunus* species. Chemical analysis of the phloem centrifugates showed that the chemical composition of trees infected with '*Ca.* P. prunorum' was indistinguishable from the composition of non-infected *Prunus* trees. In accordance, the development of *C. pruni* nymphs was not influenced by the infection of host plants.

The knowledge obtained in this thesis is essential for the development of innovative and selective control strategies against *C. pruni* based on semiochemicals, such as push-pull and attract-and-kill strategies. Further breeding programs of resistant *Prunus* cultivars should take the findings of this work into account.

Zusammenfassung

Der Pflaumenblattsauger, *Cacopsylla pruni* ist eine univoltine Insektenart, welche sich mit ihren spezialisierten Mundwerkzeugen stechend-saugend vom Phloem ihrer Wirtspflanzen ernährt. Während ihres Lebens alternieren Pflaumenblattsauger zwischen laubtragenden *Prunus*-Bäumen und immergrünen Koniferen. Zu Beginn des Frühjahrs fliegen die adulten Blattsauger in Steinobstanlagen, wo sie ihre Eier bevorzugt auf bestimmte *Prunus*-Arten ablegen. Nach der Paarung und der Eiablage sterben die Individuen der alten Generation (Remigrants). Die nächste Generation entwickelt sich von April bis Juni. Die jungen Adulten (Emigrants) bleiben noch einige Tage auf den Steinobstbäumen, bevor sie auf Nadelbäume in höheren Lagen abwandern. Dort verbleiben sie bis zum nächsten Frühjahr, in welchem sie zwecks Reproduktion wieder zurück zum Steinobst wandern.

Das Verbreitungsgebiet des Pflaumenblattsaugers erstreckt sich über Europa und angrenzende Gebiete. Von Relevanz für den Obstanbau ist *C. pruni* hauptsächlich wegen seiner Fähigkeit, das Phytoplasma '*Candidatus* Phytoplasma prunorum' zu übertragen. Dabei handelt es sich um ein zellwandloses Bakterium, welches in seinen Wirtspflanzen ausschließlich im Phloem verbreitet ist. Wenn die Pflaumenblattsauger an infizierten *Prunus*-Bäumen saugen, nehmen sie die Phytoplasmen aus dem Phloem auf. Nachdem sich die Bakterien in den Insekten vermehrt haben, können sie über den Speichel der Blattsauger auf gesunde *Prunus*-Bäume übertragen werden. '*Ca.* P. prunorum' ruft die sog. Europäische Steinobstvergilbung (European Stone Fruit Yellows, ESFY) hervor. Dabei handelt es sich um eine der bedeutendsten Pflanzenkrankheiten im Europäischen Obstanbau, welche zu massiven Ernteausfällen und wirtschaftlichen Einbußen führt. Von den typischen Symptomen, wie dem verfrühten Austrieb, dem chlorotischen Blattrollen und der Notreifung der Früchte, sind vor allem für den Anbau kultivierte Sorten von Pfirsichen (*Prunus persica*), Aprikosen (*Prunus armeniaca*) und Japanischen Pflaumen (*Prunus salicina*) betroffen. In diesen Arten führt die Infektion innerhalb weniger Jahre zum Absterben der Bäume. Heimische Arten wie Schlehen (*Prunus spinosa*) und wilde Pflaumen (*Prunus cerasifera, Prunus insititia*) zeigen meist keine schwerwiegenden Symptome, ebenso die meisten kultivierten Sorten von Pflaumen (*Prunus domestica*).

Bis heute gibt es keine Maßnahmen zur Bekämpfung von Phytoplasmosen. Eine Alternative stellt die Regulation der Vektorinsekten dar. Mit Hilfe sogenannter Infochemikalien könnte das Verhalten der Blattsauger so manipuliert werden, dass diese aus den Steinobstanlagen ferngehalten werden. Dadurch kann die Anzahl der Neuinfektionen mit ESFY gemindert werden. Da bisher nur wenig über die Biologie und Ökologie des Pflaumenblattsaugers bekannt ist, habe ich in der vorliegenden Arbeit den Einfluss von pflanzenbürtigen Duft- und Inhaltsstoffen auf das Verhalten und die Fitness von *C. pruni* untersucht.

Anhand von Feldstudien zum Vorkommen von *C. pruni* in verschiedenen *Prunus*-Arten identifizierte ich welche Wirtspflanzen von *C. pruni* bevorzugt besiedelt werden. Dadurch konnte ich *P. insititia* als eine der bevorzugten *Prunus*-Arten einstufen. Im Gegensatz dazu wurden nur wenige Pflaumenblattsauger auf *P. persica* Bäumen gefunden. In den Studien zur Wirts-Präferenz von *C. pruni* der vorliegenden Arbeit wurden die Duft- und Inhaltsstoffe dieser beiden *Prunus*-Arten und Koniferen verglichen, um den Einfluss von olfaktorischen und gustatorischen Reizen auf das Verhalten der Pflaumenblattsauger zu beurteilen. Mit der Aufzeichnung von Elektroantennogrammen konnte ich zeigen, dass Pflaumenblattsauger Weibchen sowohl volatile Substanzen aus dem Duft von *Prunus*-Bäumen als auch typische Nadelbaumdüfte wahrnehmen können. An Hand von Olfaktometerversuchen mit *C. pruni* konnte ich jedoch die Bevorzugung bestimmter Wirtspflanzen nicht allein auf olfaktorische Reize zurückführen. Daher untersuchte ich ebenfalls den Einfluss der Pflanzeninhaltstoffe auf *C. pruni*. In einer Entwicklungsstudie konnte ich beweisen, dass sich *C. pruni* Nymphen besser auf der präferierten wilden Pflaumenart *P. insititia*, als auf der weniger bevorzugten kultivierten Pfirsichsorte *P. persica* cv. South Haven entwickeln können. Die Entwicklungsunterschiede korrelieren mit den Ergebnissen meiner Untersuchung zur Zusammensetzung des Phloemsaftes beider *Prunus*-Arten. Für die Präferenz von bestimmten Wirtspflanzen scheinen gustatorische Reize für *C. pruni* wichtiger zu sein als olfaktorische Signale.

Dass die Zusammensetzung des Pflanzensaftes eine wichtige Rolle für *C. pruni* spielt, konnte ich durch weitere Entwicklungsstudien an Koniferen bestätigen. Es zeigte sich, dass sich *C. pruni* Nymphen nicht auf Nadelbäumen entwickeln können, auch wenn sie Pflanzensaft von Koniferen aufnehmen. Adulte *C. pruni* hingegen überleben signifikant länger auf Nadelbäumen als ohne Nahrungsquelle. Woraus ich schließe, dass sie die immergrünen Nadelbäume als Wasser- und Nährstoffquellen im Winter benötigen und daher auf den Wirtswechsel angewiesen sind.

Des Weiteren wurde in der vorliegenden Arbeit untersucht, ob sich eine Infektion mit '*Ca.* P. prunorum' auf die Interaktion zwischen den Vektorinsekten und ihren Wirtspflanzen auswirkt. Zu diesem Zweck wurde das Saugverhalten der Nymphen an ESFY-infizierten und nicht-infizierten *P. insititia* und *P. persica* Bäumen mittels Elektropenetrographie untersucht. Dabei zeigte sich, dass sich die Infektion der *Prunus*-Pflanzen nur auf die mittlere Dauer der Aufnahme von Xylemsaft auswirkt. *C. pruni*-Nymphen saugten durchschnittlich weniger am Xylem von infizierten *Prunus*-Bäumen. Die durchschnittliche Dauer der Saugaktivität im Phloem der Wirtspflanzen wurde nicht durch die Infektion beeinflusst. Zusätzlich analysierte ich den Inhalt des Phloems. Dabei war es nicht möglich, dessen chemische Zusammensetzung auf Grund der '*Ca.* P. prunorum' Infektion zu unterscheiden. In Übereinstimmung mit diesen Ergebnissen wirkte sich die Infektion der Wirtspflanzen nicht auf die Entwicklungsgeschwindigkeit von *C. pruni*-Nymphen aus.

Die in dieser Arbeit neu gewonnen Erkenntnisse zur chemischen Kommunikation von *C. pruni* bilden die Grundlage für die Entwicklung von innovativen und selektiven Bekämpfungsmethoden, basierend auf Semiochemikalien, wie Push-Pull-Systeme und Attract-and-Kill-Strategien. Zudem sollten die Ergebnisse bei der Züchtung von resistenten *Prunus* Sorten berücksichtigt werden.

1. Introduction

1.1. Psyllids

General

Psyllids or jumping plant lice belong to the order of Hempiptera. Today eight psyllid families are classified, which consist of about 3850 species distributed nearly all over the world (Burckhardt and Ouvrard, 2012; Hodkinson, 2009). Around 400 species are known to occur in Europe (Jarausch et al., 2019a). Some species in the genus *Cacopsylla* in the Psyllidae family colonize Rosacea species cultivated for fruit production and cause economical damage, in the main affecting apple, pear and stone fruits (Hodkinson, 2009; Jarausch et al., 2019a).

Life history

The lifecycle of psyllids consists of an egg-stage and five nymphal instars (Fig. 1c, d). Unwinged nymphs are dorsoventrally flattened and mobile (Ossiannilsson, 1992). In European *Cacopsylla* species two different life-history strategies have evolved. Some species, such as the pear psyllids *Cacopsylla pyri* and *Cacopsylla pyricola*, are polyvoltine, producing up to five overlapping generations annually and hibernate on deciduous Rosacea species (Hodkinson, 2009; Lauterer, 1999). In contrast, related species are univoltine and migrate between divergent plant species (Fig. 3). Best-known examples are *Cacopsylla picta*, *Cacopsylla melanoneura* and *Cacopsylla pruni*. These species reproduce on plants belonging the rose family, but the newly emerged adults (called emigrants) leave the trees after some days to weeks and migrate to conifers in higher regions (Hodkinson, 2009; Ossiannilsson, 1992; Thébaud et al., 2009). In early spring, the same individuals remigrate (remigrants) to rosaceous trees for mating and oviposition (Gallinger et al., 2019a; Labonne and Lichou, 2004; Mayer et al., 2011). Even though such a host alternation enables insects to avoid unfavorable environmental conditions and offers new options, migration flights are costly (Rankin, 1992). Next to energy costs for the flight, it commonly includes reproductive costs. In addition, migration behavior carries further risks, as well as the challenge of finding suitable hosts over distance (Rankin, 1992). Until today reasons and mechanisms of the host alternation of European psyllid species are under-investigated.

Phloem feeding

Insects are selective feeders. Besides the specialization to host ranges, resource specialization is a common concept in herbivorus insects. Feeding on specific plant parts and specialized feeding mechanisms can be classified in different feeding-guilds (Novotny et al., 2010). Psyllids are phytophagous hemipterans. The nymphs and the adults feed with their piercing-sucking mouthparts on the phloem sap of plants (Price et al., 2011; Schoonhoven et al., 2005). Therefore, psyllids as other phloem-feeders have to face the challenge of utilization of phloem content for nutrition. The

main function of the phloem tissue is the long-distance translocation of photoassimilates from source to sink organs in plants (Patrick, 2013). Commonly carbon is translocated in the form of sucrose, raffinose or sugar alcohols, such as sorbitol, mannitol or dulcitol (Lambers et al., 2008). In addition, organic acids including amino acids, which are the major source of nitrogen in animals, are transported through the phloem (Douglas, 2006). Nonetheless, sugars are dominating the phloem sap and amino acids are scarce. Feeding compensation occurs in phloem-feeders to ingest sufficient essential amino acids, but lead to an uptake of more carbohydrates than required (Price et al., 2011). To get rid of the surplus sugars, phloem-feeding insects excrete undigested sugars as honeydew (Douglas et al., 2006). Additionally, in the gut of psyllids and other hemipterans, several endosymbionts are found that might provide their hosts with additional nutrients (Baumann, 2005; Cooper et al., 2017; Douglas, 2003). Next to resources movement, phloem plays an important role in plant defense, as phytohormones are distributed via the phloem (see chapter 1.3). Furthermore, specialized bacteria can colonize the phloem tissue, causing severe plant diseases (see chapter 1.2). In addition to direct damage due to mass occurrence, some psyllid species harm their host plants by infecting them with phloem dwelling bacteria. One of these bacteria transmitting species is the plum psyllid *C. pruni*.

Cacopsylla pruni

Cacopsylla pruni (Scopoli, 1763) (Figure 1) migrates between *Prunus* and coniferous trees (Fig. 3) as described above and reproduces exclusively on some species belonging to the *Prunus* genus. Whereas newly emerged emigrants are light green (Figure 1e) and turn into orange to pale brown with grayish forewings after some days (Figure 1f), the overwintered remigrants are red-brown colored with characteristic dark brown forewings (Figure 1a, b). *C. pruni* is abundant in stone fruit growing areas all over Europe (Fialová et al., 2004; Fialová et al., 2007; Jarausch et al., 2008; Jarausch et al., 2019b; Jarausch et al., 2019a; Labonne and Lichou, 2004; Maier et al., 2013; Mergenthaler et al., 2017; Sabaté et al., 2016; Yvon et al., 2004). In field surveys diverging preferences for different *Prunus* species and genotypes are reported. Commonly *C. pruni* is most abundant on *Prunus spinosa* and *Prunus cerasifera*, varying numbers are found on *Prunus domestica* genotypes (Labonne and Lichou, 2004; Mergenthaler et al., 2017). In Spain, high numbers are also captured in wild *Prunus mahaleb* (Sabaté et al., 2016). *C. pruni* is able to survive on *Prunus amygdalus*, *Prunus armeniaca*, and *Prunus persica* (Carraro et al., 2004a), but field studies monitored less individuals on species cultivated for fruit production, such as apricots (*P. armenica)*, peaches (*P. persica)* and Japanese plums (*P. salicina)* in the field (Mergenthaler et al., 2017; Warabieda et al., 2018). Nonetheless plum psyllids cause severe damage to these fruit crops, because they transmit the pathogen '*Candidatus* Phytoplasma prunorum' (Jarausch et al., 2008; Jarausch et al., 2019a).

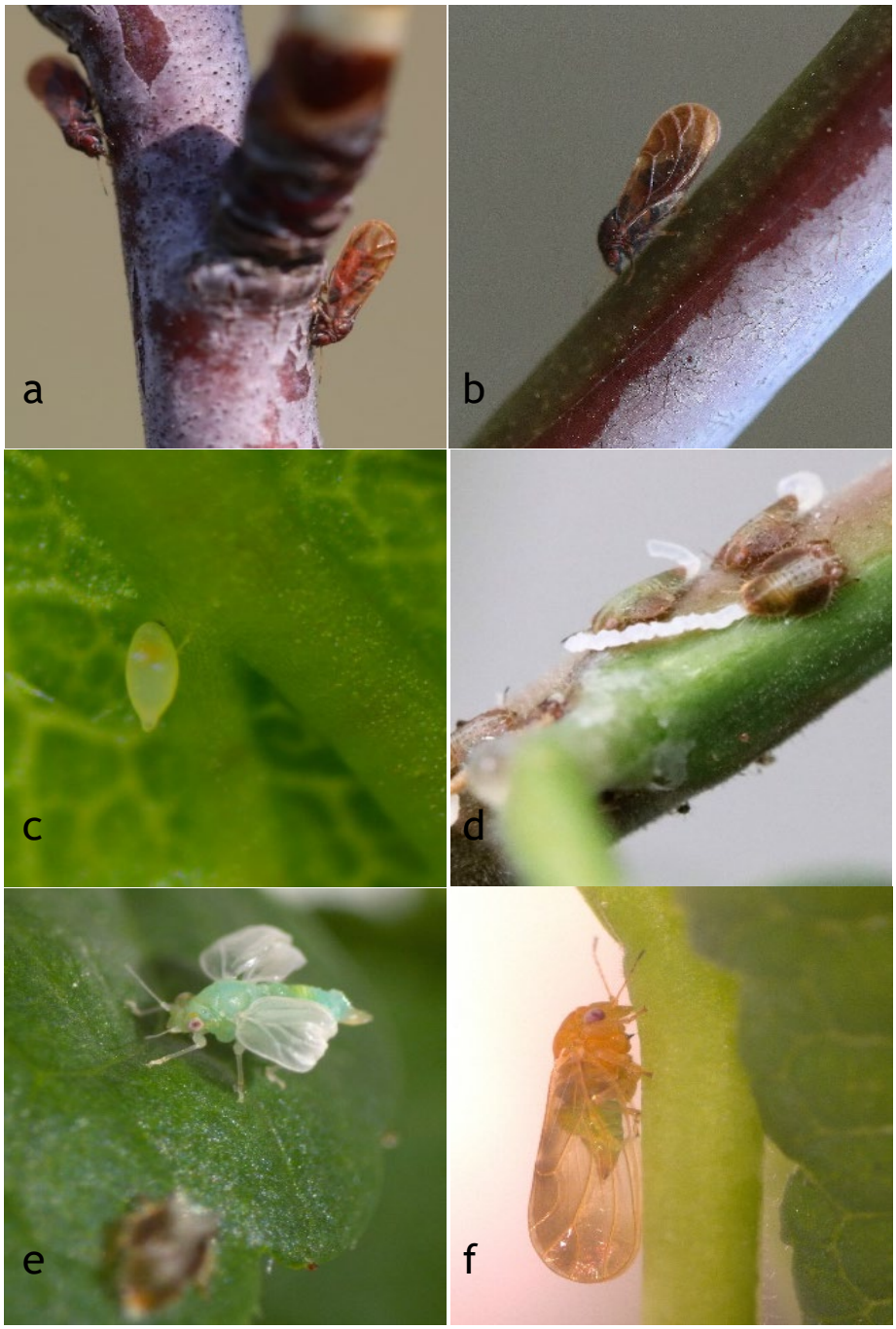

Figure 1: Developmental stages of *Cacopsylla pruni*: a) overwintered female remigrants; b) male remigrant; c) egg on the abaxial leaf surface; d) 5th instar nymphs on a lateral branch producing honeydew; e) newly emerged adult female; f) 3 day old female emigrant.

1.2. Phytoplasma

General

Phytoplasmas are cell wall-less obligate parasitic bacteria, causing severe diseases in plants. They have small linear chromosomes and show limited function of metabolic pathways. Therefore, they rely on their host for nutrition (Bai et al., 2006; Kube et al., 2008; Marcone et al., 1999). In their

specific host plants, they are restricted to the phloem tissue (Pagliari and Musetti, 2019). Sap sucking insects, such as leafhoppers, planthoppers, cicadas and psyllids, acquire the bacteria during feeding at the phloem of infected plants (acquisition feeding). If insects ingest sufficient amount of phytoplasmas they are able to establish in the vector. During the latent period the pathogens invade the insect gut, move into the haemocoel and multiply. When phytoplasmas insert the salivary glands, infected insects are able to transmit the pathogen to healthy plants through their saliva during phloem-feeding (inoculation feeding) (Carraro et al., 1998; Hogenhout et al., 2008; Thébaud et al., 2009).

'*Ca.* P. prunorum' and European stone fruit yellows

Lorenz et al. (1994) discovered that previously known yellowing diseases of several stone fruits such as apricot chlorotic leafroll (ACLR), plum leptonecrosis (PLN), peach yellowing and plum decline are caused by the same phytoplasma and introduced the name European stone fruit yellows (ESFY) phytoplasma. Ten years later Seemüller and Schneider (2004) revealed the close relationship between apple proliferation (AP), pear decline (PD) and European stone fruit yellows phytoplasmas, belonging to the apple proliferation group (16SrX) and proposed the name '*Ca.* P. prunorum' for the causal agent of ESFY. In contrast, the so-called peach X-disease, distributed in North America, is caused by '*Candidatus* Phytoplasma pruni', belonging to a different phytoplasma subgroup (Lee et al., 2000; Lorenz et al., 1994). The most characteristic symptoms of ESFY are the enlargement of midribs and swollen main lateral veins as well as chlorotic leafroll and yellowing/reddening of leaves (Figure 2). In some species off-season growth and a premature bud brake has been recorded after ESFY infections (Lorenz et al., 1994; Marcone et al., 2010; Smith, 1997). Japanese plums infected by vector transmission show an incubation period of 4-5 month before first symptoms are visible (Carraro et al., 1998). Infected apricots are reported to die within 12-24 month after appearance of symptoms (Smith, 1997). Additionally, fruit set and pollen germination in some diseased apricots cultivars is decreased, indicating a loss in fruit yield (Nečas et al., 2017). In general severity of disease and symptom manifestation is variable, depending on the susceptibility of *Prunus* species and genotype as well as phytoplasma strain virulence (Kison and Seemüller, 2001; Koncz et al., 2017; Richter, 2002). Although infected wild *P. spinosa*, *P. cerasifera* commonly remain symptom free, these plants may harbor '*Ca.* P. prunorum' and represent a pathogen reservoir (Carraro et al., 2002). ESFY is reported from nearly all apricot and peach growing areas in central and southern Europe: Germany (Jarausch et al., 2008; Jarausch et al., 2019b), France (Jarausch et al., 2001; Thébaud et al., 2006; Yvon et al., 2004), Austria (Laimer Da Câmara Machado et al., 2001), Switzerland (Ramel and Gugerli, 2004), Italy (Marcone et al., 1996; Poggi Pollini et al., 2007; Poggi Pollini et al., 2010), Spain (Sabaté et al., 2016; Torres et al., 2004), Hungary (Koncz et al., 2017; Mergenthaler et al., 2017; Tarcali et al., 2014), Romania, Slovenia (Steffek et al., 2012) and

the Czech Republic (Fialová et al., 2004; Fialová et al., 2007; Nečas et al., 2017). In Poland '*Ca.* P. prunorum' is present but actually not rated as a dangerous threat to Polish stone fruit production (Warabieda et al., 2018). ESFY phytoplasma has also been detected in *Prunus* cultivars in Azerbaijan (Balakishiyeva et al., 2010), Turkey (Ulubaş Serçe et al., 2006) and in the African countries Egypt (Steffek et al., 2012) and Tunisia (Khalifa et al., 2011). There is evidence that '*Ca.* P. prunorum' is transovarial transmitted within *C. pruni* (Tedeschi et al., 2006). Transmission trails revealed that both adults and nymphs are able to transmit the phytoplasma (Carraro et al., 1998). 1st instar nymphs acquired the bacteria after 2 to 4 days on infected plants followed by a varying latency period that lasted at minimum 2 weeks (Carraro et al., 2001). The bacteria are persistent in *C. pruni. T*herefore, infected individuals that migrate to *Prunus* after overwintering are very infectious (Carraro et al., 2001; Carraro et al., 2004b; Thébaud et al., 2009). A study on the presence of '*Ca.* P. prunorum' in flowers, fruits, seedlings and pollen indicate no bacterial transmission by seeds or pollen (Nečas et al., 2017).

Figure 2: *P. persica* plant and leaf a) without ESFY and b) infected with '*Ca.* P. prunorum', showing characteristic leaf yellowing and reduced growth.

Crop protection measurements - current situation

Crop protection measurements against phytoplasmas do not exist. Attempts to cultivate phytoplasmas in artificial media still fails, impeding the development of cures. In the European Union ESFY and further phytoplasma associated diseases are quarantine (Smith, 1997) and regulated in the Council Directive 2000/29/EC (Council of the European Union, 2000). Today the use of verified healthy rootstocks and cultivars as well as clearing of infected trees are the only possible phytosanitary measures to prevent the spread of ESFY. Alternatively, an effective strategy for vector control could help to reduce the pathogen spread. In 2015 the only approved insecticide against *C. pruni* in

Germany expired the authorization for application (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2018). The control of *C. pruni* with insecticides cypertmethrin (pyretroid) and thiacloprid (neonicotinoid) was very effective, but came along with risks of those non-selective chemicals for pollinators and other beneficial insects (Paleskić et al., 2017). Therefore, the development of eco-friendly plant protection measurements should be the future aim. A selective and environmentally friendly control strategy could be based on semiochemicals (Gross and Gündermann, 2016).

1.3. Chemical communication

General

The metabolism of chemical substances and the development of chemical sense in plants and insects enables these organisms to interact with each other and their environment. Chemicals that release behavioral responses in intra- and interspecific communications are also referred to as semiochemicals. Pheromones are semiochemicals used in intraspecific interactions (Karlson and Lüscher, 1959). Insects for example, use pheromones to find mating partners, to mark trails to food sources or to alert conspecifics to threats, such as predators (Regnier and Law, 1968; Schoonhoven, 1990). The communication between individuals from different species is based on metabolites called allelochemicals. Nordlund and Lewis (1976) categorized these chemicals in regard to their impact on the emitter and receiver. While allomones are beneficial for the emitter but have adverse effects in the receiver, kairomones are detrimental for the emitter but have advantages for the receiver (Nordlund and Lewis, 1976). Common examples for allomones are plant volatiles that repel or nonvolatile substances that deter phytophagous insects and save the plant from herbivore attack. In contrast, kairomones are e.g. plant volatiles that attract phytophagous insects to their hosts (Price et al., 2011). Signals that benefit the emitter as well as the receiver, such as plant volatiles elicited after herbivore attack that attract predators or parasitoids, are defined as synomones (Price et al., 2011).

Host finding and acceptance by herbivores

Insects show different degrees of host specializations. Many herbivorous insects are specialized to a narrow range of host plants (monophagous and oligophagous), which they accept for feeding and oviposition (Bernays and Graham, 1988). Therefore, the detection of suitable host plants is crucial for survival and reproductive success and phytophagous insects developed sophisticated chemoreceptors to locate and identify their hosts due to volatile and nonvolatile metabolites. In general, visual and olfactory cues are important for the orientated movement (searching behavior) over

distance (Deletre et al., 2016; Schoonhoven et al., 2005). Commonly, herbivorous insects use blends of several plant-emitted volatiles to identify and locate suitable hosts (Bruce and Pickett, 2011). After landing on the plant, physical factors of the surface and gustatory cues become important for host plant evaluation (Schoonhoven et al., 2005; Visser, 1988). Insects can use information from nonvolatile secondary plant metabolites, such as epicuticular waxes on the plant surface that can promote acceptance of host plants for feeding and oviposition (Müller and Riederer, 2005; Powell et al., 1999; Rid et al., 2018). Additionally, primary metabolites affect host selection. The content of primary metabolites enables insects to recognize food quality, which is for this very reason important for food selection process. Gustatory receptor neurons located on the mouthparts and tarsi enable insects to perceive information from the plant surface and the inside of the plant tissue (Chapman, 2003). The carbohydrates sucrose and fructose act as feeding stimulants for phytophagous insects (Mittler and Dadd, 1963; Arn and Cleere, 1971). Phagostimulatory neurons that respond to sorbitol are detected in caterpillars of Lepidopteran species specialized to Rosacea, as sorbitol is a characteristic metabolite in rosaceous plants (Chapman, 2003; Ziegler and Mittler, 1959). Lapointe et al. (2016) elaborated a three-component blend that stimulates the stylet penetration of Asian Citrus Psyllid, *Diaphorina citri*. In contrast, deterrents inhibit feeding or oviposition. Some phenolic compounds are shown to act as antifeedants for hemipterans (Dreyer and Jones, 1981; Grayer et al., 1994), but specific compounds that deter psyllids from feeding are not yet identified.

Plant defense

Several defense mechanisms have evolved in plants, enabling the sessile organisms to defend themselves against attacking herbivores and microbes. Some morphological and chemical mediated protections are constitutive in plants while others are produced only in response to insect feeding or infestations with microbial pathogens (Baker et al., 1997; Chisholm et al., 2006; Karban and Baldwin, 1997; War et al., 2012). For example, the production of allomones, which repel or deter attacking herbivores, as well as synomones, which indirectly defend the plant by luring predators or parasitoids of the herbivores, can be induced (Schoonhoven et al., 2005). An example of widedistributed physical defense are trichomes, the formation of which is constitutive but can also be increased in response to herbivory (Dalin et al., 2008). Induced plant responses are regulated by phytohormones, signal molecules that regulate physiological and metabolic processes in plants. Plant responses towards biotic stress appear diverse and complex and depend on the type of enemy and attack, as well as on the degree of damage and the wounded plant part. Two main pathways are induced by enemy attack depending either on jasmonic acid (JA) or salicylic acid (SA). Chewing-biting herbivores cause severe physical damage to plant tissue. Next to wound responses, as reaction to the mechanical wounding, molecules in regurgitates and saliva from insects are recognized

by the plant and elicit specific immune reactions (Felton and Tumlinson, 2008; Walling, 2000). Receptors in the plant bind the elicitors and induce the production of jasmonates that activate specific defense mechanisms, such as the production of specific volatiles, alkaloids, trichomes or extrafloral nectar (Heil and Ton, 2008). Salicylic acid plays a major role in plant defense against biotrophic pathogenic microorganisms, such as fungi, viruses and bacteria, depending on living plant tissue (Ma and Ma, 2016; Robert-Seilaniantz et al., 2011).

Similar pathways are activated by piercing-sucking herbivores, causing minimal and localized injury to the plant tissue. Elicitors in the saliva of phloem-feeding insects, or microbes induce plant defense mechanisms such as sieve tube occlusion (Chisholm et al., 2006; Will et al., 2013; Will and van Bel, 2006). In some cases, the SA as well as the JA pathway is activated. For example, pathogens or microbes from the plant surface can attach to herbivores and may enter into plant tissue during herbivore feeding (Felton and Tumlinson, 2008).

Information about induction of phytohormones in response to psyllid attack is rare. No literature is available on the phytohormone concentrations in Rosacea after *Cacopsylla* infestation. Nehela et al. (2018) revealed higher concentrations of auxins, SA, JA and abscisic acid (ABA) in leaves from *Citrus sinensis* trees infested with *D. citri* compared to leaves from non-infested trees. Ibanez et al. (2019) confirmed the accumulation of SA and SA metabolites in *C. sinensis* leaves in response to long time infestations with *D. citri*. To date the number of studies on the role of the phytohormones (ethylen, abscisic acid, auxins and cytokinins) on plant defense mechanisms and the crosstalk of different hormones are rising (Robert-Seilaniantz et al., 2011; Thaler et al., 2012), illustrating the complex interplay between herbivores, microorganisms and plants.

The transmission of '*Ca.* P. prunorum' by *C. pruni* causes severe threats to several cultivated stone fruit crops. Studies focusing on the epidemiology of ESFY and the abundance of the vector in fruit orchards are dominating the literature, but little is known about the biology of the vector insect. A broad knowledge about *C. pruni* could help to develop specific, innovative and sustainable plant protection measurements and lower the spread of ESFY.

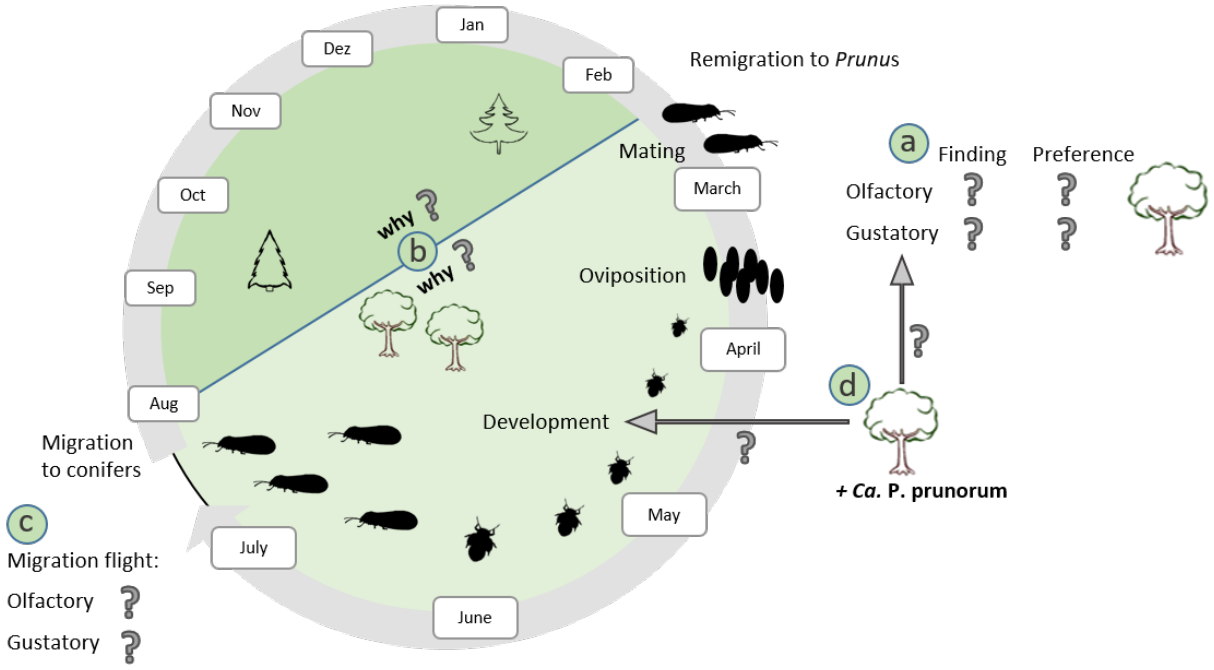

Figure 3: Life cycle of *C. pruni* and influence of olfactory and gustatory senses on different life cycle aspects on deciduous and coniferous trees and during migration that were investigated in this thesis: a) host finding and preference, b) migration between conifers and stone fruit trees, c) triggering migration flight, d) phytoplasma infection of host trees.

Therefore, I focused my research on the impact of infochemicals on several life cycle aspects of *C. pruni* and the role of '*Ca.* P. prunorum' in plant – insect interaction (Fig. 3).

I addressed the following research questions

- Which chemical cues influence the host finding and host preferences of *C. pruni* (Fig. 3a)?
- Why is *C. pruni* migrating between *Prunus* and conifers (Fig. 3b)?
- Are volatile organic compounds influencing the migration behavior (Fig. 3c)?
- Which impact has an infection of *Prunus* trees by '*Ca.* P. prunorum' on the feeding behavior and development of *C. pruni*? Are the behavior and development influenced by gustatory cues (phloem chemistry) (Fig. 3d)?

2. Summary of publications

Publication 1

Collection, Identification, and Statistical Analysis of Volatile Organic Compound Patterns mitted by Phytoplasma Infected Plants

Jürgen Gross, Jannicke Gallinger and Margit Rid

Published 2019 in: Musetti R, Pagliari L (eds) Phytoplasmas: Methods and Protocols. Springer, New York, pp 333–343

The analysis of volatile organic compounds (VOCs) is an important tool for chemical ecologists. In this protocol, we present headspace sampling methods and give advice for the chemical analysis of VOC samples and the handling and evaluation of measured data. The chapter focuses on the special case of comparison of VOC compositions from phytoplasma infected and non-infected host plants. The described approach in general is certainly suitable for different kind of studies investigating volatile metabolites from the atmosphere.

Different devices for headspace sampling and ideal accessories are listed that minimize contaminations and allow exact and comparable sampling. The use of a portable sampling device, developed by the working group, is described in more detail. The chemical analysis by gas chromatography followed by mass spectrometry (GC-MS) is recommended and the identification and quantification with AMDIS software (Automated Mass Spectral Deconvolution and Identification System) is explained in detail. Appropriate multivariate statistical methods to visualize and calculate similarities and differences in compositional data are shortly introduced and discussed. Additionally, we provide an R-script to convert the AMDIS output in a compositional dataset that is needed for further statistical analysis.

The described procedure of headspace sampling and analysis was used for the comparison of VOC patterns emitted from different host plants of *C. pruni* reported in publication 2. In addition, the identification and quantification with AMIDS and the statistical evaluation of GC-MS data were used for the analysis of phloem and xylem content compositions in publication 3 and 4.

Publication 2

Host Plant Preferences and Detection of Host Plant Volatiles of the Migrating Psyllid Species *Cacopsylla pruni*, the Vector of European Stone Fruit Yellows

Jannicke Gallinger, Barbara Jarausch, Wolfgang Jarausch, Jürgen Gross

Published 2019 in: *Journal of Pest Science*

Cacopsylla pruni migrates between *Prunus* trees for reproduction and coniferous trees for overwintering. Hence, *C. pruni* needs to locate host plants of divergent classes over great distance. Insects commonly use volatile signals to locate their host over distance. The detection and reaction of *C. pruni* towards VOCs from *Prunus* and coniferous hosts was investigated in this study. It was hypothesized that volatile signals could trigger the migration behavior, if *Prunus* VOCs are repellent and odors from conifers are attractive for young adults (emigrants) and vice versa for remigrants. Additionally, monitoring studies report differences in the abundance of *C. pruni* on several *Prunus* species. Thus far, the impact of plant volatiles on this preference of *C. pruni* for some *Prunus* species over others and on the migration behavior was not investigated before.

In this study, we monitored the preference of *C. pruni* for different *Prunus* cultivars by beating tray method in the field over three years, to identify favored *Prunus* species. We caught high numbers of *C. pruni* from *P. spinosa*, *Prunus besseyi* × *P. cerasifera* and *P. insititia*. We showed a low abundance of *C. pruni* on *P. persica* and *P. armeniaca* trees and confirmed them as non-preferred hosts of plum psyllids. We choose to sample the headspace of *P. insititia* cv. 'GF655-2', which was considered as an attractive cultivar and the non-attractive *P. persica* cv. 'South Haven'. In addition, volatile samples were collected from silver firs (*Abies alba*) exemplary for conifers as overwintering hosts. VOCs were sampled directly in the field (as described in publication 1) at several developmental stages of the plants. In total, we determined 114 VOCs and compared their composition in the headspace of the three host species at different phenological stages. Aldehydes (hexanal, octanal, nonanal, decanal, dodecanal), the ketone 1-phenylethane-1-one and the alkane tridecane were identified to be characteristic for *Prunus* odor profiles. Especially at early developmental stages of the plants high proportions of these compounds contributed to the volatile composition. Their relative amount decreased over time, because green leaf volatiles (GLV) (Z)-3-hexene-1-ol and (Z)-3-hexenyl acetate dominated the odor samples with progressing leaf development. Overall, low relative abundances of terpenes were detected in samples from both *Prunus* species. In contrast, the following terpenes were characteristic for the odor of silver firs: camphene, myrcene, terpinolene, alpha-pinene and limonene. Another characteristic compound in silver fir samples was bornyl

acetate, the acetate ester of the terpene borneol. We found no differences in the odor profiles from *A. alba* between the two sampling times (spring and fall). We tested the detection of the characteristic VOCs by *C. pruni* females with electroantennography. All selected aldehydes except dodecanal and all tested terpenes released a significant antennal response. Additionally, the psyllid antenna reacted to the application of (Z)-3-hexenyl acetate and 1-phenylethane-1-one. The application of dodecanal, (Z)-3-hexene-1-ol, tridecane and bornyl acetate did not elicit receptor potentials greater than solvent application. At least we investigated the preference of *C. pruni* remigrants and emigrants for the different host plants in olfactometer trails. Contrary our expectations, remigrants did not prefer *P. insititia* plants over *P. persica* plants, if plant odors are offered simultaneously in an y-shaped olfactometer. Furthermore, neither remigrants nor emigrants showed a preference for *P. insititia* or *A. alba* odors if offered simultaneously.

Our field survey proved that *C. pruni* has divergent preferences for *Prunus* species and our EAG study confirmed that *C. pruni* is able to detect characteristic host plant volatiles. Nonetheless, the preferences for different *Prunus* species are not mediated by plant odors alone. Additional cues, such as visual and / or gustatory stimuli must have some influence on plant preference of *C. pruni.* Even though *C. pruni* females detected VOCs from coniferous plants as well as from *Prunus*, which enables them to locate their desired hosts for reproduction and overwintering. The migration flight of *C. pruni* seems not to be triggered by changing attractiveness or repellence of plant odors depended on the age of *C. pr*uni. We conclude that other factors, such as gustatory cues, play an important role in host acceptance and migration behavior of *C. pruni.* Therefore, the impact of the chemical composition of host plant phloem and xylem was investigated in the following publications 3 and 4.

Publication 3

Unraveling the Host Plant Alternation of *Cacopsylla pruni* – Adults but Not Nymphs Can Survive on Conifers Due to Phloem/Xylem Composition

Jannicke Gallinger, Jürgen Gross

Published 2018 in: *Frontiers in Plant Science*

To unravel the reasons for migration of plum psyllids between conifers and *Prunus* trees, we investigated the feeding behavior, development and survival of *C. pruni* on different host plants in this study. We recorded electrical penetration graphs (EPG) of emigrants and fifth instar nymphs on several coniferous trees and *P. domestica* cv. Wavit. With these recordings, we were able to demonstrate that a migrating psyllid species actually feeds on conifer diet. In accordance with this, the bioassays revealed that newly emerged emigrants survive on *P. abies* and *A. alba* as long as on *P. domestica*. On all plants, they survived significantly longer than without food supply. This results demonstrate that *C. pruni* adults rely on coniferous diet to survive the winter. EPG studies further showed that also nymphs penetrate conifer needles and ingest plant saps. We therefore concluded that conifer volatiles do not repel *C. pruni* nymphs and no mechanical barriers hinder them to feed on conifers. If *C. pruni* feeds on conifers, the question arises why they need to migrate to *Prunus*.

We therefore investigated the development of nymphs on *P. domestica*, *P. abies* and *A. alba* We found that although *C. pruni* nymphs show feeding behavior on conifers, they were not able to develop from second instar to adult stage on these plants, as all nymphs died on the investigated conifers. In contrast, 92% adults eclosed from nymphs developed on *P. domestica*. To identify components that could affect the feeding and development of *C. pruni* the phloem and xylem sap was extracted from *A. alba*, *P. abies*, *Larix decidua*, *Pinus sylvestris* and *P. domestica* trees by centrifugation technique. We derivatized sugars, sugar alcohols, amino acids and other organic acids in plant sap centrifugates and analyzed the samples via GC-MS. Afterwards we compared the composition of these metabolites between the different plant species (as described in Pub.1). The sugar alcohol sorbitol was the main compound in samples from *P. domestica*. In contrast, we did not detect sorbitol in sap samples from conifers, which contained great proportions of pinitol instead. Caffeic acid and a great proportion of asparagine was characteristic for *P. domestica* saps. In contrast, conifer plant saps contained no caffeic acid but high proportions of quinic acid. On one hand sorbitol or caffeic acid, which were exclusively found in *Prunus* sap samples, could act as phagostimulants. On the other hand, compounds in coniferous diet could act as deterrents for *C. pruni* nymphs.

Publication 4

Phloem Metabolites of *Prunus* sp. rather than Infection with '*Candidatus* Phytoplasma prunorum' Influence Feeding Behavior of *Cacopsylla pruni* Nymphs

Jannicke Gallinger, Jürgen Gross

Published 2020 in: *Journal of Chemical Ecology*

Previous studies (Pub. 2 and 3) imply that phloem sap chemistry affects host preference, feeding behavior and development of *C. pruni*. As *C. pruni* is the only known vector of '*Ca.* P. prunorum', we were interested how the infection of *Prunus* trees with '*Ca.* P. prunorum' influences the vector fitness. Additionally, in publication 2 we were not able to show that olfactory cues are responsible for host plant preferences of *C. pruni* and therefore hypothesized a great impact of gustatory cues. To elucidate the role of phloem chemistry for host acceptance and performance of *C. pruni* we compared the feeding behavior and development of nymphs on two host plants of different attractiveness: *P. insititia* as a preferred and *P. persica* as a non-favored host plant species. This preference was detected in previous field surveys (Pub. 2).

First, we investigated the development of nymphs on ESFY-infected and non-infected *P. insititia* and *P. persica* plants. Interestingly the phytoplasma infection of both *Prunus* species had no impact for the development of *C. pruni*. In contrast, their development was significantly elongated and less successful on *P. persica* compared to that on *P. insititia*. Less adults developed from nymphs reared on *P. persica* trees, only 12 % on non-infected and 15 % on infected plants. In contrast, four times more adults eclosed on *P. insititia* trees, 57 % on non-infected and 60 % on ESFY-infected trees. The evaluation of occurrence and duration of several waveforms (feeding behaviors) from EPG recordings revealed a reduced phloem feeding of nymphs on *P. persica* plants, which explains the reduced development. On average nymphs fed three times longer on the phloem of *P. insititia* than on *P. persica*. From a total time period of 16 h nymphs on *P. persica* spend 13 % of the time with phloem ingestion and 9 % with ingestion of xylem content. Nymphs feeding on *P. insititia* ingested phloem on average for 40 % and xylem for 7 % of the time. In contrast to these great differences in the duration of feeding, the frequency of occurrence and the time until the first phloem ingestion occurs was not different between the plant species. Therefore, it was concluded that reduced feeding time was caused by the chemical composition of phloem sap, rather than morphological differences between the plants. The phloem sap content of infected and non-infected *P. insititia* and *P. persica* trees was sampled by centrifugation technique. After derivatization of plant metabolites, the samples were analyzed via GC-MS. Contrary to our expectations, the

infection with '*Ca.* P. prunorum' did not change the metabolic composition of phloem centrifugates neither from *P. persica* nor *P. insititia* plants. Instead, the comparison of phloem chemistry revealed significant differences between the *Prunus* species.

3. Discussion

Very few information is available about the chemical communication of plum psyllids with their environment, even though the spreading of '*Ca.* P. prunorum' is a severe threat for fruit growing with a great economic impact. For the development of innovative crop protection measurements, a detailed knowledge about the biology and ecology of target pests is crucial. Therefore, I focused my work on the identification of chemical cues that shape the behavior of *C. pruni* (Fig. 4), which can be used for the design of ecofriendly control strategies based on semiochemicals.

Olfaction: Host preference of *C. pruni* is not the result of plant volatiles alone.

We were able to show that *C. pruni* detects characteristic volatiles from different host plants and identified characteristic volatile compounds for coniferous overwintering host and alternate reproduction host trees (*Prunus*), which are detectable by *C. pruni* antenna (Pub. 2). These compounds can act as kairomones that enable plum psyllids to locate and may distinguish their host plants during migration flight. Nonetheless, behavioral studies elucidated that volatiles are less important for host acceptance (Pub. 2, Fig. 4a). Even though we found differences in the odor composition between more and less attractive *Prunus* cultivars, the content of EAG active components seems to depend on the developmental stage of the plants rather than the *Prunus* species (Pub. 2). I conclude that olfactory cues play a minor role for host plant preference of *C. pruni.* That host selection of psyllids is not based on plant volatiles alone is also found for other psyllid species (Farnier et al., 2018; Horton and Landolt, 2007; Soroker et al., 2004; Wenninger et al., 2009). This is in accordance with the general assumption, that olfactory signals are important for host searching over distance, but gustatory and textual cues on the plant surface and in the plant tissue play the major role in host acceptance (Schoonhoven et al., 2005). The great importance of gustatory and mechanosensory information for host acceptance is well documented for other phloem-feeding insects, manly aphids (Douglas, 2003). Whereas information about the sensory mediated choice of psyllids is still rare. Patt et al. (2011) highlighted the interaction and synergistic effects of olfactory, visual and gustatory stimuli on psyllid behavior. Additionally, the colonization of Asian Citrus Psyllid (*D. citri)* of preferred plant parts (young flush) is influenced by nutritional factors as well as morphological parameters (George et al., 2017; Sétamou et al., 2016). These findings imply the great importance of phloem-sap ingredients and composition on psyllid settling and feeding behavior.

Gustation: Phloem content chemistry influences the vector development and host plant preference of *C. pruni*.

In our study *C. pruni* nymphs showed increased feeding activity on preferred *P. insititia* plants over *P. persica* trees. These differences in behavior are correlated with significant differences in the composition of the phloem sap of both *Prunus* species (Pub. 4). Comparable results were found in

behavioral studies with *C. pyricola* suggesting that cues perceived from the leaf surface and from inside the plant tissue affect the feeding behavior and oviposition of the psyllid and lead to acceptance or rejection of plants as hosts (Horton and Krysan, 1991; Ullman and McLean, 1988). Indicating that gustatory stimuli play a major role in host acceptance and preference in psyllids (Fig. 4a). In general, the morphology of psyllid mouthparts is similar to other Hemipterans. Even though there are no studies on the gustatory receptors on the feeding apparatus of *C. pruni* existing, different types of chemosensory sensilla are described from the mouthparts of related pear psyllid species *C. pyricola* and *C. chinensis* (Forbes, 1972; Liang et al., 2013; Ullman and McLean, 1986). Garzo et al. (2012) reported labial sensilla of Asian Citrus Psyllids and hypothesized a gustatory sensory function in comparison to aphid sensilla. The identification of feeding-stimulants for *D. citri* provided evidence for the importance of gustation for psyllid behavior (George et al., 2016; Lapointe et al., 2016; Patt et al., 2011). As a result of the increased phloem ingestion of *C. pruni* nymphs on *P. insititia*, nymphs had a greater development success on *P. insititia* compared to *P. persica* (Pub. 4). This finding strongly supports the hypothesis that *C. pruni* is well adapted to indigenous European *Prunus* species, such as *P. insititia*.

Significance of plant chemistry for migration: *C. pruni* needs *Prunus* trees for development and conifers as food source in winter.

Behavioral studies with remigrants and emigrants from the related species *C. melanoneura* and *C. picta* indicated that olfactory cues may trigger migration behavior of psyllids (Mayer et al., 2011). In contrast, the preference for coniferous and *Prunus* host plants does not change due to developmental stage of *C. pruni* (Pub. 2, Fig. 4c). Considering that plant VOCs do not elicit the migration flight of *C. pruni,* two new questions arise: what causes the migration and why do plum psyllids alternate their hosts. Many psyllid species have a narrow host range (Hodkinson, 2009). Thus, the development of alternation between such diverged tree species is of high interest. The strong impact of phloem chemistry of *Prunus* host on feeding behavior and fitness of *C. pruni* suggest the assumption that phloem content is also important for host alternation. Our studies revealed that *C. pruni* is not able to develop on coniferous trees (Pub. 3). Nymphs are maybe adapted to *Prunus* diet and therefore reject feeding on conifers, as early life stages are commonly sensitive to plant quality (Schoonhoven et al., 2005). In contrast, survival of *C. pruni* adults is possible on coniferous diet (Pub. 3). Although psyllids are said to be phloem-feeders, EAG studies reveal that they are not only ingesting phloem sap notably adults ingest less phloem but more xylem compared to immature psyllids (Ebert et al., 2018; George et al., 2018), demonstrating that adults need less nutrients. In accordance with our findings, the ingestion of xylem sap might be essential for psyllid fluid balance. I conclude that adult *C. pruni* feed on phloem and xylem of coniferous trees for water and nutrient uptake as coniferous trees provide enough energy and water for overwintering of adults, but not

for development of nymphs. Therefore, plum psyllids need to migrate to evergreen conifers to survive during winter and need to remigrate to stone fruit trees, because reproduction is impossible on conifers (Fig. 4b).

All results of this study imply that gustatory cues have a great impact on psyllid behavior, thus the release of migration flight could be triggered by seasonal changes in phloem of host plants. A similar mechanism is detectable in aphids. Some species evolved strategies to avoid their woody hosts during mid-summer (Moran, 1992; Sandström, 2000). For example, *Brachycaudus helichrysi, Brachycaudus cardui*, *Hyalopterus pruni* and *Myzus persicae* are aphid species that migrate from their primary *Prunus* hosts *(P. domestica* and *P. persica* resp.) to secondary host in summer (Jousselin et al., 2010; Latham and Mills, 2011; Shim et al., 1977). The reasons for this migration behavior of aphids are still unclear. Sandström (2000) suggested that mature woody plants are unfavorable hosts for aphids, but was not able to attribute the poor suitability to nutrient composition. As *C. pruni* also starts its migration flight in the beginning of summer (Jarausch et al 2019a), similar unknown reasons may trigger this early start of migration behavior. Differences in nutritional quality and leaf anatomy of host should be investigated by seasonal analysis of *Prunus* phloem.

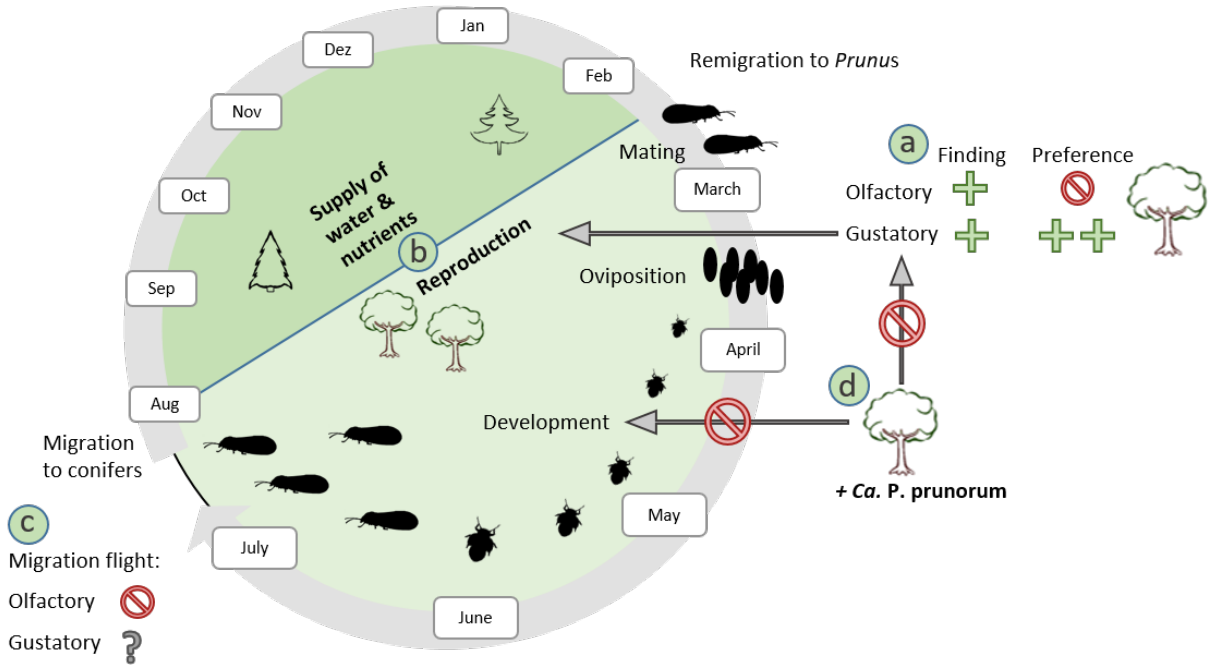

Figure 4: Impact of olfactory and gustatory cues on the lifecycle of the plum psyllid *Cacopsylla pruni.*

Impact of phytoplasma on the plant – insect interaction: No impact on phloem composition and development of *C. pruni*.

Due to the importance of gustatory stimuli on psyllid behavior, we concentrated our studies on the influence of phytoplasma infections on the content of vascular tissue of *Prunus* trees. We expected

that the phytoplasma infection manifest in differences in the composition of phloem sap content of *P. insititia* and *P. persica* plants. It is known that phytoplasma infections negatively affect the photosynthetic activity, plant metabolism and change the translocation of metabolites in infected plants (Bertamini et al., 2002; Bertamini et al., 2004; Christensen et al. 2005; Maust et al., 2003). Altered metabolite distribution in plants could be related to disturbed hormone balance in plants (Dermastia, 2019). Contrary to our expectation, we were not able to distinguish the phloem sap composition between infected and non-infected *Prunus* trees (Pub. 4). This might be due to an antagonistic crosstalk between induced phytohormones, SA and jasmonic acid iso-leucin (JA-Ile), the major bioactive conjugate of JA (Staswick and Tiryaki, 2004). SA and JA-Ile concentrations were significantly increased in leaves of ESFY-infected *P. persica* plants (unpublished data). Phytoplasma-triggered changes in phytohormone levels are also reported for apple trees infected with '*Ca.* P. mali' (Zimmermann et al., 2015). Janik et al. (2017) revealed that differences of SA, JA-Ile and ABA levels between infected and non-infected apple trees are changing over time. Reciprocal antagonistic effects between SA and JA are well studied in model plants Arabidopsis, tomato and tobacco (Thaler et al., 2012). For example, the single application of JA and SA affected the composition of phloem sap of *Plantago lanceolata* plants, but when both phytohormones had been applied at the same time, these effects disappeared (Schweiger et al., 2014). Additionally, and in contrast to single application, the survival of aphids was not reduced by the simultaneous application of both hormones (Schweiger et al., 2014). In accordance to previously stated, we found no impact on the development of *C. pruni* due to ESFY infections of *P. persica* and *P. insititia* plants in our study (Pub. 4, Fig. 4d). Contrasting results were documented for the related species *C. picta,* which offspring has a decreased development success on phytoplasma infected apple trees (Mayer et al., 2011). It is still unknown whether the '*Ca.* P. mali' infection affects the *C. picta* progeny due to changes of phloem composition (food quality) or other factors such as disturbed phloem anatomy or defense mechanisms, such as callose deposition. The impairment of the vascular system, due to anatomical changes might be responsible for a number of symptoms, such as chlorosis, leaf yellowing, swollen leaf-veins and curly of leaves, that are among others characteristic for ESFY-infected peach trees. Even though the vascular tissue of *Prunus* trees might be affected by the '*Ca.* P. prunorum' infection, such anatomical changes do not negatively affect the feeding behavior of *C. pruni* nymphs. The latter indicates that plant defense mechanisms induced in response to the phytoplasma infection are not efficient to defend the plants against the insect *C. pruni.*

Evolution of the plant-pathogen-vector system

Tedeschi and Bertaccini (2019) concluded a long-term coevolution of '*Ca.* P. prunorum' and its vector based on the fact that '*Ca.* P. prunorum' is vertically transmitted to *C. pruni* progeny (Tedeschi et al., 2006). Our finding that the phytoplasma infection does not negatively affect the

vector development supports this statement. *C. pruni,* as well as '*Ca.* P. prunorum' are considered to be indigenous to Europe. *P. insititia* is closely related to domesticated *P. domestica* and wild plums *P. cerasifera* (Zohary et al., 2012). Therefore, *P. insititia, P. domestica* and *P. cerasifera* are accepted to be autochthonous to Europe. In contrast *P. persica, P. armeniaca* and *P. salicina* have their origin in Asia. As no wild ancestors can be found in Europe, it is hypothesized that they have been introduced as already domesticated cultivars to Europe (Huang et al., 2008; Zohary et al., 2012). Our developmental studies demonstrate the well-established adaptation of *C. pruni* to European *P. insititia* in contrast to the less suitable Asian host *P. persica* (Pub. 4).

In addition, many studies on the epidemiology of ESFY already highlighted the differences in the sensitiveness of European and Asian *Prunus* species to the pathogen. In general, European *Prunus* are more tolerant, whereas Asian *Prunus* species suffer severely from ESFY infections. Studies on the reproduction success/fitness of *C. pruni* on further species of Asian origin and the impact of '*Ca.* P. prunorum' infections on plant defense mechanisms in European *Prunus* could further elucidate the adaption of both, the vector and the phytoplasma to European *Prunus* species.

Conclusion & Outlook

This thesis contributes to the knowledge about the biology of *C. pruni.* Increasing knowledge can guide the development of alternative strategies, to control the vector and reduce the spread of ESFY. As a main result of this doctoral thesis, gustatory cues are very important for host plant selection of *C. pruni*. Therefore, the influence of the compounds found in the phloem of host plants on the feeding behavior needs to be in the focus of further experiments with artificial diets. Volatiles that are detectable by *C. pruni* were identified in this work. Even though the behavioral activity of volatiles alone was less than expected, their capability for psyllid behavior manipulation has to be investigated perhaps in combination with visual stimuli. The identification of gustatory and olfactory attractants and phagostimulants can be used for innovative and selective attract-and-kill protection measurements against plum psyllids. The identification of volatiles that mask host odors or repel *C. pruni* (Gallinger et al. 2019b) can be combined with lure-baited traps to push-pull strategies. In addition, these findings might be transferable to other psyllid species, which are vectors of various phytoplasma diseases, such as apple proliferation and pear decline. The results presented in this work highlight the importance of the coevolution of the plant-pathogen-insect interaction, which has to be considered in future studies, management plans and breeding programs of different *Prunus* species.

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5. Publications

Publication 1

Collection, Identification, and Statistical Analysis of Volatile Organic Compound Patterns Emitted by Phytoplasma Infected Plants

Jürgen Gross, Jannicke Gallinger and Margit Rid

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Host Plant Preferences and Detection of Host Plant Volatiles of the Migrating Psyllid Species *Cacopsylla pruni*, the Vector of European Stone Fruit Yellows

Jannicke Gallinger, Barbara Jarausch, Wolfgang Jarausch, Jürgen Gross

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JGa and JGr designed the study. JGa, JGr, BJ and WJ contributed to the interpretation of the data, approved the final version of the manuscript and ensured the accuracy and integrity of the work. BJ and WJ conducted the field monitoring. JGa conducted the EAG and olfactometer experiments, headspace analysis and wrote the first draft of the manuscript. The manuscript was revisited and edited by BJ, WJ and JGr. JGr supervised the project

Publication 3

Unraveling the Host Plant Alternation of *Cacopsylla pruni* – Adults but Not Nymphs Can Survive on Conifers Due to Phloem/Xylem Composition

Jannicke Gallinger and Jürgen Gross

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Unraveling the Host Plant Alternation of *Cacopsylla pruni* – Adults but Not Nymphs Can Survive on Conifers Due to Phloem/Xylem Composition

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Plant sap feeding insects like psyllids are known to be vectors of phloem dwelling bacteria ('*Candidatus* Phytoplasma' and '*Ca*. Liberibacter'), plant pathogens which cause severe diseases and economically important crop damage. Some univoltine psyllid species have a particular life cycle, within one generation they alternate two times between different host plant species. The plum psyllid *Cacopsylla pruni*, the vector of European Stone Fruit Yellows (ESFY), one of the most serious pests in European fruit production, migrates to stone fruit orchards (*Prunus* spp.) for mating and oviposition in early spring. The young adults of the new generation leave the *Prunus* trees in summer and emigrate to their overwintering hosts like spruce and other conifers. Very little is known about the factors responsible for the regulation of migration, reasons for host alternation, and the behavior of psyllids during their phase of life on conifers. Because insect feeding behavior and host acceptance is driven by different biotic factors, such as olfactory and gustatory cues as well as mechanical barriers, we carried out electrical penetration graph (EPG) recordings and survival bioassays with *C. pruni* on different conifer species as potential overwintering hosts and analyzed the chemical composition of the respective plant saps. We are the first to show that migrating psyllids do feed on overwintering hosts and that nymphs are able to ingest phloem and xylem sap of coniferous trees, but cannot develop on conifer diet. Analyses of plant saps reveal qualitative differences in the chemical composition between coniferous trees and *Prunus* as well as within conifer species. These differences are discussed with regard to nutritional needs of psyllid nymphs for proper development, overwintering needs of adults and restriction of '*Ca.* P. prunorum' to *Prunus* phloem.

Keywords: phloem, chemical composition, psyllid, development, overwintering, host alternation, migration, conifer

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INTRODUCTION

Phloem and xylem tissue enables plants to allocate their resources from sources to sinks and distribute phytohormones to regulate physiological processes. Especially the phloem is rich in nutrients (Douglas, 2006), making it a suitable food source for sap-sucking insects. Although mechanical barriers like sclerenchymatous fibrous rings are able to hinder phloem-feeders from

reaching the vascular bundles (George et al., 2017), the phloem is poorly chemically defended (Douglas, 2006). Since decades studies focused on the chemical composition of phloem sap and the nutrition of phloem-feeding insects. Most work was done in the field of crops, such as rice (Fukumorita and Chino, 1982), broad bean, clover, and peas (Sandström and Pettersson, 1994; Wilkinson and Douglas, 2003) and their pests (especially aphids), because of the economic importance and the role of aphids as model organisms. Information about the composition of phloem and xylem sap of coniferous plants is rare. Ziegler and Mittler (1959) extracted phloem sap from *Picea abies* by stylectomy and found sucrose as the only sugar in paper chromatography analysis. Later studies focused on induced defense mechanisms in bark phloem after bark beetle attack (Rohde et al., 1996), food quality of needles (Schopf et al., 1982; Fisher and Fisher, 1987) and impact of air pollution on nutrition of conifers (Zedler et al., 1986; Kainulainen et al., 1993). These studies give an impression of which metabolites could be found in plant sap of coniferous trees, but compounds were extracted from whole plant tissue (bark resp. needles). More explicit knowledge about plant sap composition is important for a better understanding of the biology of phloem-feeding insects that migrate between two different host plant species, e.g., psyllids (Hemiptera: Psyllidae).

Psyllids or jumping plant lice are plant sap feeding insects encompassing more than 3000 species. Most of them are oligophagous and use perennial dicotyledonous angiosperms as host plants for reproduction (Hodkinson, 2009; Mayer et al., 2009, 2011). In the genus *Cacopsylla* two different strategies can be observed: There are polyvoltine species reproducing and feeding exclusively on the same host plant and univoltine species with an obligate alternation of two host plants (Ossiannilsson, 1992; Hodkinson, 2009). The latter migrate between their reproduction host plants (respective fruit crops) and their overwintering host plants (conifers) (Mayer and Gross, 2007; Mayer et al., 2011). For identifying their particular host plants for feeding and reproduction, volatile signals are used in many species during migration (Soroker et al., 2004; Gross and Mekonen, 2005; Mayer et al., 2008a,b, 2009; Weintraub and Gross, 2013).

The plum psyllid, *Cacopsylla pruni* is the only known vector of one of the most serious pests in European fruit production, the cell wall lacking bacterium '*Candidatus* Phytoplasma prunorum' (Carraro et al., 1998). The phloem dwelling bacterium induces the European Stone Fruit Yellows (ESFY) (Seemüller and Schneider, 2004). Because infected trees yield poorly and die quickly, this plant disease causes high economic losses in European fruit production every year. So far no curative approach was found against this disease. Unfortunately, it is not possible to cultivate this obligate cell parasite outside of the host plant or vector, which hampers research toward a cure. Therefore, the only measure to inhibit infection of stone fruit orchards is to prevent invasion of the vector insect, as *C. pruni* alternates between *Prunus* spp. and coniferous trees during its life cycle. After reproduction and development on *Prunus* spp., the young adults (emigrant stage) emigrate and spend the rest of the year on spruce and other conifers (Thébaud et al., 2009; Jarausch and Jarausch, 2016). In early spring they return to *Prunus* spp. for reproduction (remigrant stage). Very little is known about the reason for migration and feeding behavior of psyllids during their life on conifers (Thébaud et al., 2009). To date it remains unclear whether overwintering psyllids actually feed on conifers. Former experiments with the closely related hawthorn psyllid *Cacopsylla melanoneura* failed, although the maintenance of body condition and level of hydration suggested feeding (Jackson et al., 1990). Because it was shown that adult *C. pruni* did not survive the winter on one of their reproduction hosts *Prunus spinosa* (Carraro et al., 2002; Thébaud et al., 2009), and that some migrating species including *C. pruni* already start migration to their overwintering host during summer (Mayer and Gross, 2007; Mayer et al., 2009; Jarausch and Jarausch, 2016), we hypothesize that *C. pruni* needs to feed on overwintering host plants during this long period and therefore needs to leave deciduous *Prunus* trees to migrate to evergreen conifers, which show yearlong photosynthesis and phloem activity. On the other hand, reproduction on coniferous trees could be impossible for *C. pruni*, forcing them to migrate back to *Prunus*. A better knowledge of the vector biology is needed to develop new control strategies against vector insects and bacterial pathogens (Gross and Gündermann, 2016; Perilla-Henao and Casteel, 2016).

Here, we studied the feeding behavior of adults and nymphs on several conifer species as well as *Prunus domestica*, and conducted bioassays to unveil *C. pruni*'s ability to survive and develop on plant sap of overwintering hosts. Furthermore, we extracted the phloem/xylem sap of both *Prunus* spp. and conifers and analyzed sugars and organic acids including amino acids.

MATERIALS AND METHODS

Insects

Cacopsylla pruni remigrants (overwintered adults) were caught by beating tray method from *Prunus domestica* trees located at the experimental field of the Julius Kühn-Institut in Dossenheim, Germany and at an experimental orchard of Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Neustadt an der Weinstrasse, Germany in spring 2017. Psyllids were maintained on *Prunus* trees (cv. GF655/2 and *Prunus spinosa*) in cages housed in a climate chamber at 20°C during photophase and 16°C during scotophase (L16:D8). After mating and oviposition the field captured adults were transferred to cages with fresh plants. For survival experiments about 200 fifth instar nymphs were gently transferred to a new *P. domestica* (cv. Wavit) tree and emerged adults (emigrants) were collected daily.

Plants

Four conifer species, *Abies alba* (Silver fir), *Larix decidua* (European larch), *Picea abies* (Norway spruce), and *Pinus sylvestris* (Scots pine), and the *P. domestica* cultivar Wavit were used for experiments. Plants were grown under natural conditions in an insect safe environment. Hexythiazox (Ordoval, BASF, Ludwigshafen am Rhein, Germany) and Fenpyroximate (Kiron, Cheminova Deutschland GmbH & Co.

KG, Stade, Germany) were applied once to *P. domestica* plants in April 2017 to prevent infestation with spider mites.

EPG-Recordings

To investigate whether *C. pruni* adults and nymphs feed on coniferous trees in general, the electrical penetration graph technique (EPG) was applied. EPGs were recorded using an 8 channel amplifier (model Giga-8d, EPG-Systems, Wageningen, Netherlands). Data acquisition and analysis was performed with Stylet+ software (EPG-Systems). To connect the psyllids to a copper electrode, a piece of fine gold wire (18 µm) was attached to the pronotum with a small droplet of water based silver glue (EPG-Systems). The electrode was attached to an EPG probe and the reference electrodes were placed in the soil of the test plants. Feeding behavior of *C. pruni* male and female emigrants (minimum age 6 weeks) was recorded in a climate chamber at 10°C with 60–65% RH for 16 h and of fifth instar nymphs (about 6 weeks old) at 20°C under the same conditions. Plants and insects were housed in a grounded self-constructed Faraday cage during recordings. Recordings were replicated 10 times for nymphs on each *P. abies*, *A. alba,* and *P. domestica* (cv. Wavit). Feeding behavior of emigrants was recorded on *P. sylvestris* (4 males and 6 females), *P. abies* (6 males and 4 females), *A. alba* (5 males and 5 females), and *L. decudia* (6 males and 4 females). To ensure that emigrants used for EPG recordings were not repelled by conifers (due to their developmental stage), *C. pruni* adults were caged with *P. abies* and *A. alba* twigs one day prior recordings and only emigrants which were found on conifer twigs were chosen for the experiment. Recordings were examined for occurrence of stylet penetration and waveforms indicating phloem and xylem uptake according to Bonani et al. (2010) and Civolani et al. (2011).

Bioassays

Survival

Survival of emigrants was studied on *P. abies*, *A. alba*, and *P. domestica* cv. Wavit plants. Transparent plastic cups (0.5 l capacity) were used as cages. The bottom of each cup was replaced by gauze for venting. A hole was punched into the lids to attach the cups on twigs of living plants. The lid was sealed with self-made modeling clay (composed of 42.6% water, 42.6% flour, 3.2% sunflower oil, 10.6% salt, and 1.1% citric acid) and five newly emerged emigrants (<24 h) were released in each cup. Living individuals were recorded daily over a period of 40 days. Additionally, the mortality of emigrants in the same type of cups, but without food supply (control), was observed. The experiment was replicated eight times for every plant species and five times without plants (control) under rearing conditions.

Development

For developmental experiments *C. pruni* nymphs of second and third instar were gently transferred with a fine brush from rearing plants to twigs with young flush of *P. abies*, *A. alba*, or *P. domestica* cv. Wavit, respectively. On each plant, five nymphs were caged in insect rearing sleeves (40 cm × 20 cm, 100 × 80 mesh/square inch, MegaView, Taiwan). The experiment was replicated seven times on each conifer species and five times on cv. Wavit. Experimental plants were housed under rearing conditions in a climate chamber. After 21 days cages were controlled consistently once a week for hatched *C. pruni* adults (emigrants). After 56 days all cages were opened and checked for living nymphs.

Xylem and Phloem Sap Sampling

Phloem and xylem saps were collected in June 2017 using modified centrifugation technique according to Hijaz and Killiny (2014). The twigs from young flush from *P. domestica* (cv. Wavit) and conifer species *P. abies, A. alba, L. deciduas,* and *P. sylvestris* were sliced into 2–3 cm pieces with a clean scalpel. The bottom of a 0.5 ml Eppendorf tube was removed with a razor blade and twig pieces were placed into the tube. The tube was immersed in a 1.5 ml tube. For collecting the phloem and xylem sap, the tubes were centrifuged at 12.000 rpm at 4°C for 10 min. The collected samples were stored at −80°C up to analysis. In the following, this collected mixture of phloem and xylem sap is referred as plant sap.

Plant Sap Derivatization

The sap samples were derivatized with methyl chloroformate (MCF) to focus the GC-MS analysis on amino and other organic acids (Smart et al., 2010). An aliquot of 20 µl plant sap was mixed with 180 µl sodium hydroxide (1 M) in a silanized glass vial. Then 167 µl methanol and 34 µl pyridine were added, followed by 20 µl MCF. The sample was vortexed for exactly 30 s, additionally 20 µl MCF were added and the sample was mixed again for 30 s. To extract the alkylated derivatives 150 µl chloroform were added to each sample and mixed for another 10 s. A 200 µl aliquot of sodium bicarbonate solution (50 mM) was added and mixed for 10 s again. After a double meniscus was formed, the aqueous phase was discarded and a few milligrams of anhydrous sodium sulfate were added to the organic layer to bind the remaining water. The supernatant was transferred to a GC-MS vial with a glass insert.

For the derivatization with trimethylsilyl (TMS) 5 µl aliquots of the sap samples were added to 60 µl of an internal standard solution (Ribitol in ultrapure water) and dried under nitrogen stream (Reacti-Vap, Thermo Fisher Scientific Inc., Waltham, MA, United States). Samples were derivatized by adding 70 µl methoxyamine hydrochloride solution (MOX) in pyridine (2%) and allow to incubate for 90 min at 37°C stirring at adjustment of 7 (Reacti-Therm, Thermo Fisher Scientific Inc.). 90 µl of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were added and the silylation was allowed to react for 60 min at 37°C stirring at adjustment of 7 (Reacti-Therm, Thermo Fisher Scientific Inc.). The supernatant was transferred to a GC-MS vial with a glass insert.

Chemical Analysis

Derivatized samples were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using a PerkinElmer Clarus R 680 GC system coupled to a PerkinElmer quadrupole inert mass selective detector for molecular structure analysis.

A non-polar Elite-5MS (Crossbond 5% diphenyl-95% dimethyl polysiloxane, PerkinElmer) capillary column (30 × 0.25 mm id × 0.25 µm film thickness) was used for GC separation. Carrier gas flow rate (Helium, Linde, Germany) was about 5 ml/min (column head pressure 150 kPa). Injection of 1 µl of the samples derivatized with MCF was done at 290°C injector temperature with a split flow of 1 ml/min. The initial oven temperature of 70°C was held for 3 min, followed by a temperature increase of 20°C/min up to 240°C held for 3.5 min and a further increase to 300°C at a rate of 20°C/min. The final temperature of 300°C was held for 2 min. The GC temperature program to analyze samples after silylation was as follows: the initial oven temperature of 80°C was held for 3 min, followed by an increase of 5°C/min up to 320°C. The final temperature of 320°C was held for 4 min. One microliter of each sample was injected at 220°C with a split flow of 5 ml/min. Transfer line and ion source temperatures were set to 250°C and 180°C, respectively. The quadrupole mass detector was operated in electron-impact (EI) mode at 70 eV. All data were obtained by collecting the full-scan mass spectra within the range of 35–550 m/z. Blank samples, reference standards and mixtures of alkanes (C8–C20 and C10–C40) were analyzed additionally according to both methods.

Identification and Quantification With AMDIS

GC-MS chromatograms were analyzed using "Automated Mass spectral Deconvolution and Identification System" (AMDIS, V. 2.71; National Institute of Standards and Technology NIST, Boulder, CO, United States). Detected compounds were identified by comparing characteristic ion fragmentation patterns, retention times and retention indices with standard compounds according to Weintraub and Gross (2013). For quantification, the peak areas were integrated after deconvolution with AMDIS. Identification criteria were applied as follows: match factor had to be ≥80% and the relative retention index deviation ≤5% from reference value. The settings for deconvolution were: component width: 32; adjacent peak subtraction: one; resolution: medium; sensitivity: medium; shape requirements: high; level: strong; maximum penalty: 20, and

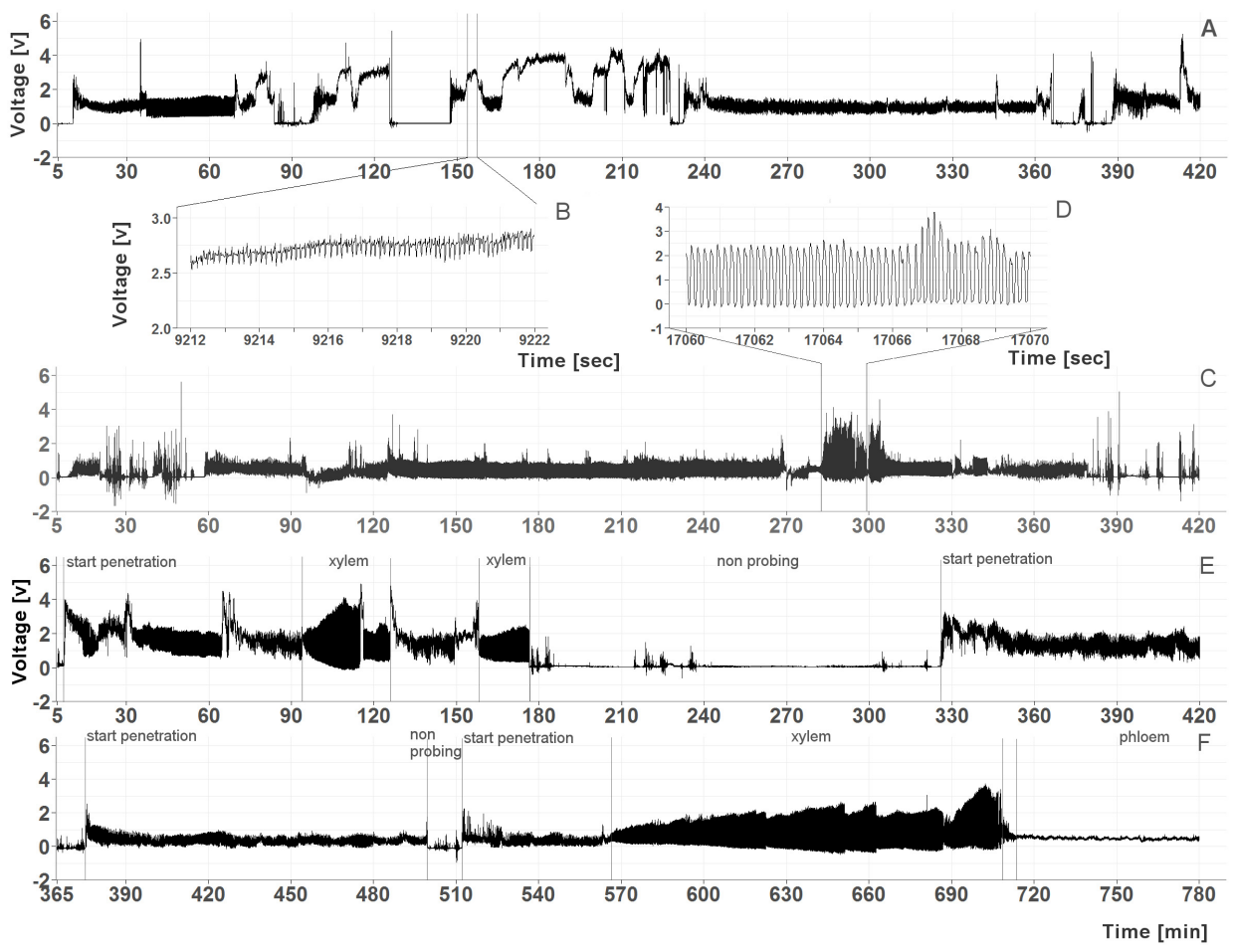

FIGURE 1 | (A–D) Examples of EPG recordings from *C. pruni* nymphs (5th instar) on spruce **(A)** with a detailed magnification of phloem phase waveform **(B)** and on fir **(C)** with a detailed magnification of the waveform of xylem feeding **(D)**. **(E,F)** Examples of recordings from a female *C. pruni* emigrant on larch **(E)** and a male emigrant on fir **(F)** with marked penetration and feeding phases.

'no RI in library': 20. Methionine, threonine, and serin were only found in traces (match < 80) and were therefore excluded from the analysis. Relative proportions of amino and organic acids were calculated by setting the sum of the selected compounds as 100%. Proportions of detected compounds after TMS derivatization were normalized to internal standard.

Chemicals and Standards

Alanine, aspartic acid, cysteine, glutamic acid, histidine, leucine, lysine, proline, threonine, tryptophan, valine, salicylic acid, pyridine, methanol, chloroform, methyl chloroformate (MCF), sodium bicarbonate, sodium sulfate, methoxyamine, ribitol, myo-inositol, xylose, pinitol, and iso-leucine were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany). Arginine and phenylalanine were purchased from SERVA Electrophoresis GmbH (Heidelberg, Germany). Glycine, methionine, serine, malic acid, caffeic acid, succinic acid, arabinose, and saccharose from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Asparagine, mannitol, glucose, and galactose from Merck KGaA (Darmstadt, Germany). Sorbitol and glutamine from AppliChem GmbH (Darmstadt, Germany). MSTFA from Macherey-Nagel GmbH & Co. KG (Düren, Germany). Citric acid was purchased from Acros Organics (Thermo Fisher Scientific, Geel, Belgium).

Statistical Analysis

Statistical analysis was done in R version 3.4.2 "Short Summer" (R Core Team, 2017). Visualizations were conducted with the ggplot2 package (Wickham, 2009). Death hazard from *C. pruni* emigrants on different host plants were compared by Cox's proportional hazard regression through likelihood ratio test. Efron approximation was used for tie handling. The proportional hazards assumption for Cox regression model fit was confirmed using the *cox.zph* function of the survival package (Therneau, 2017). Non-metric multidimensional scaling (NMDS) plots were used to visualize Bray–Curtis dissimilarities of the chemical composition of xylem and phloem between plant species. NMDS was performed using the *metaMDS* function from vegan package (Oksanen et al., 2017). Scaling was standardized by Wisconsin double standardization. Significantly ($p < 0.01$, $N = 10000$) influential factors (chemical compounds) were plotted as arrows in NMDS plots. Dissimilarity matrix was calculated to test for discrimination of plant species by Permutational Multivariate Analysis of Variance (PERMANOVA). Additionally, the dispersion of groups was tested for multivariate homogeneity (PERMDISP).

RESULTS

EPG-Recordings

To determine if *C. pruni* feeds on overwintering hosts (conifers), feeding behavior of emigrants was recorded on potential host plants. The recordings revealed that both male and female emigrants fed on plant saps of all four offered conifers: *P. abies*, *A. alba, P. sylvestris,* and *L. decudia*. Recordings from nymphs of *C. pruni* showed that they were also able to feed on *P. abies* and *A. alba* (**Figure 1**).

Bioassays

Survival

Newly emerged *C. pruni* emigrants survived on *P. abies* and *A. alba* as long as on *P. domestica* cv. Wavit (**Figure 2**). The Cox regression model showed that death hazard differed significantly between host plants and controls without food supply (likelihood ratio = 81.76, $df = 3$, $R^2 = 0.431$, $p < 0.001$). Death hazard for emigrants fed on *P. domestica* cv. Wavit did not differ from

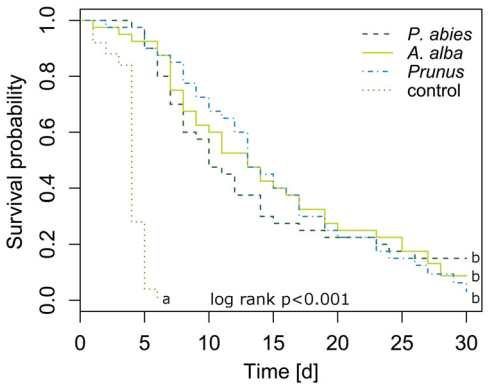

FIGURE 2 | Kaplan–Meier curves visualizing the survival of newly emerged emigrants caged on *P. abies* ($n = 40$), *A. alba* ($n = 40$), *P. domestica* cv. Wavit ($n = 40$), or in cages without a plant (control, $n = 25$). Letters indicate significant differences between survival curves (likelihood ratio = 81.76, $df = 3$, $R^2 = 0.431$, $p < 0.001$).

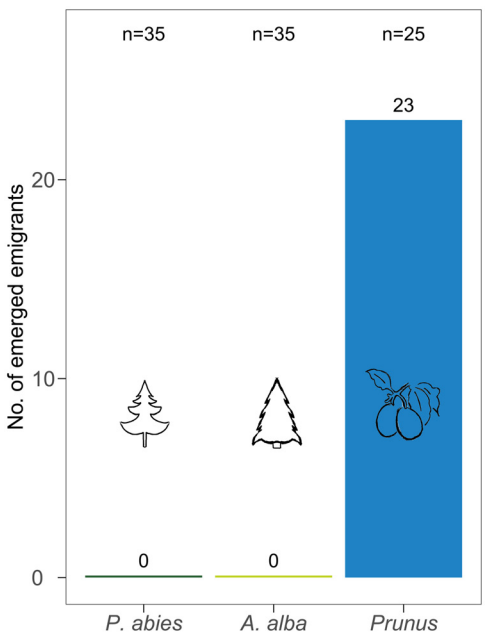

FIGURE 3 | Number of emerged *C. pruni* emigrants from nymphs (2nd instar) on *P. abies*, *A. alba*, and *P. domestica* cv. Wavit.

P. abies (likelihood ratio = 81.76, df = 3, R^2 = 0.431, p = 0.803) and *A. alba* (likelihood ratio = 81.76, df = 3, R^2 = 0.431, p = 0.846). Emigrants on all three potential host plant species had a significant lower death hazard than psyllids without food (control). The hazard ratio was reduced by 97, 97, and 96% if *C. pruni* was allowed to feed on *P. abies*, *A. alba*, or *P. domestica* cv. Wavit, respectively.

Development

After 56 days 92% of the *C. pruni* nymphs on *P. domestica* cv. Wavit emerged while none of the nymphs developed neither on *P. abies* nor *A. alba* (**Figure 3**). As no living nymphs could be found on the coniferous trees, we conclude that they all died in nymphal stage.

Chemical Composition of Phloem and Xylem Content

Plant species differed significantly in the chemical composition of sugars and other compounds detected by GC-MS analysis after TMS derivatization of plant sap (PERMANOVA, df = 4, R^2 = 60.83, N = 10000, $P < 0.001$). The dispersions differed not significantly between the groups (PERMDISP, df = 4, F = 0.42, N = 10000, $P > 0.05$), confirming that separation of species was due to their location. The NMDS plot illustrates the differences of chemical profiles (**Figure 4**).

Plant saps from *P. domestica* trees contained a high amount of sorbitol. This sugar alcohol constituted about 58% of the plant sap from *P. domestica* cv. Wavit but was not detected in samples from coniferous trees (**Figure 5**). In contrast, pinitol was exclusively found in plant sap from conifers. However, the most abundant component was quinic acid in all conifer samples (**Figure 5**). The relative abundance of quinic acid ranged from 30% in pine to 56% in spruce. Sap samples of *P. domestica* were composed of 80% sugars and sugar alcohols and 18% acids, whereas spruce, fir, pine, and larch samples consisted of 29, 41, 50, and 36% sugars and sugar alcohols and 69, 53, 43, and 61% acids, respectively.

The composition of amino acids and other organic acids differed significantly between the plant species (PERMANOVA, df = 4, R^2 = 46.85, N = 10000, $P < 0.001$). The dispersions between the groups also differed significantly (PERMDISP, df = 4, F = 3.96, N = 10000, $P < 0.01$), indicating that the separation of the plant species could be effected by different variation within species (**Figure 6**). The NMDS plot shows caffeic acid and asparagine contributing to the separation of *P. domestica* cv. Wavit from coniferous trees (**Figure 6**). Caffeic acid was exclusively found in *P. domestica* cv. Wavit, while asparagine was more abundant in *P. domestica* cv. Wavit as in *P. abies* and *A. alba* (**Figure 7**).

The main organic acid component in the plant sap of all tested plant species was malic acid (29–48%). Aspartic acid was the second most abundant component in all plants, except in larch which contained more glutamic acid. Differences between the plant species were detected concerning the relative amounts of lysine in the plant sap composition. Lysine represented about 17% of the sap samples of spruce trees and was the third most abundant component in those trees, as glutamic acid was in fir

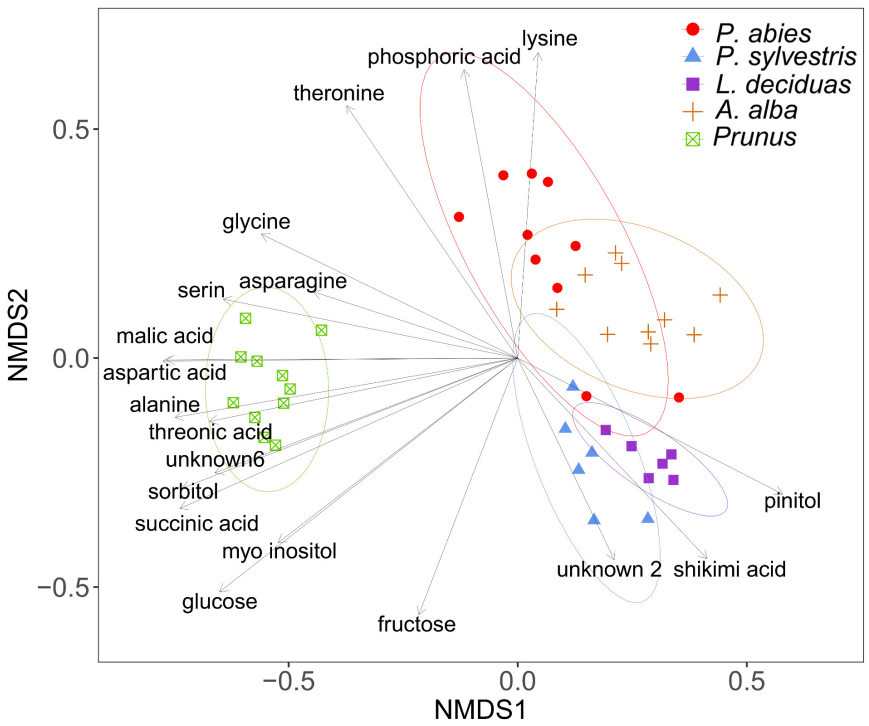

FIGURE 4 | Visualization of Bray–Curtis dissimilarities with non-metric multidimensional scaling (NMDS) plots (stress = 0.14) of plant sap samples from spruce (n = 10), pine (n = 6), larch (n = 6), fir (n = 10), and *P. domestica* cv. Wavit (n = 11) after methoximation followed by trimethylsilylation.

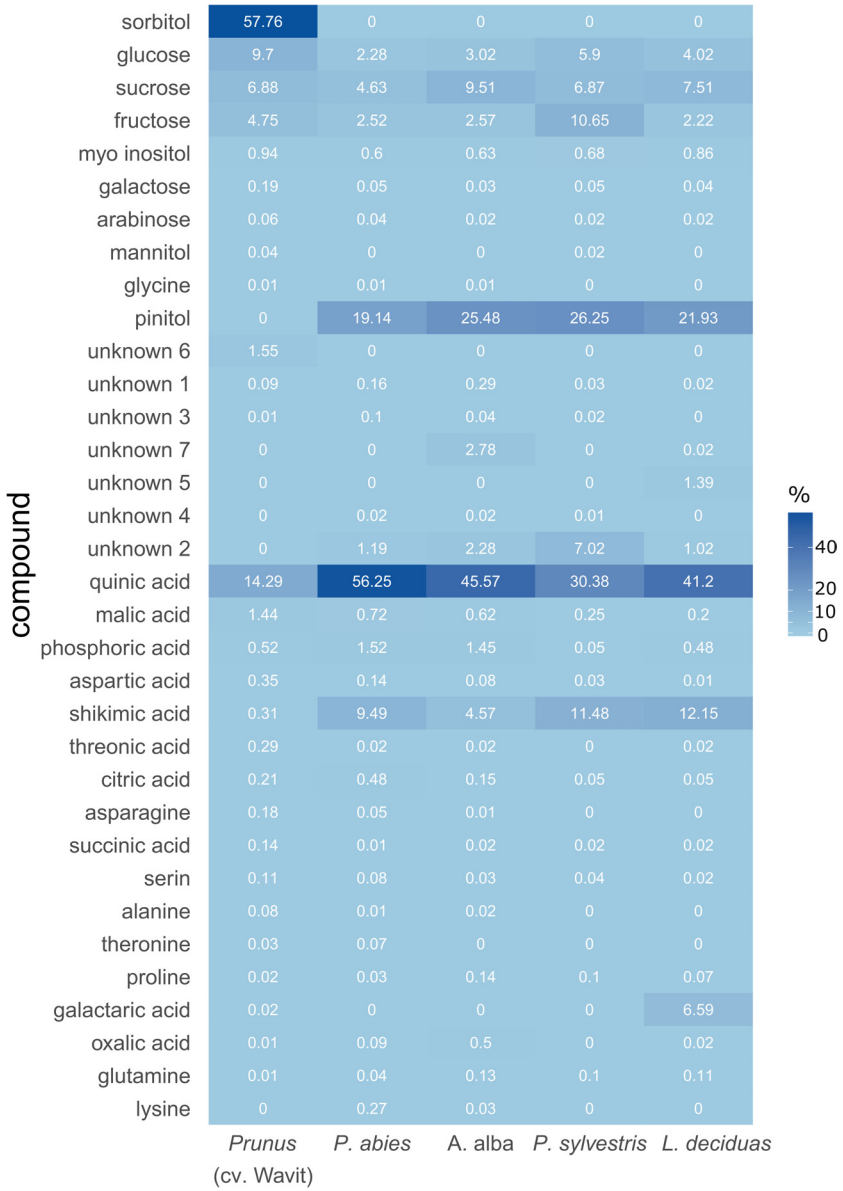

FIGURE 5 | Composition of sugars and acids in vascular bundle content of *P. domestica* cv. Wavit (n = 11), spruce (n = 10), fir (n = 10), pine (n = 6), and larch (n = 6). Plant sap was collected by centrifugation and derivatized by trimethylsilylation after methoximation. Dark blue indicates a high relative abundance of the components, light blue a low abundance. Numbers are mean values of relative abundance.

(10%), pine (15%), and *P. domestica* cv. Wavit (12%) (**Figure 7**). Cysteine, methionine, and threonine were under detection limits in all samples. The NMDS plots indicate the responsibility of the essential amino acids tyrosine, tryptophan, lysine, and histidine on the separation of spruce and fir from *P. domestica* cv. Wavit (**Figure 6**).

DISCUSSION

Electrical penetration graph recordings showed that *C. pruni* emigrants and nymphs are able to feed on the plant saps of spruce, pine, larch, and fir. EPGs recorded from 5th instar nymphs prove that nymphs are not repelled by metabolites of coniferous plants and able to reach the phloem and xylem tissue with their stylet. The question arises why *C. pruni* migrates to *Prunus* for reproduction when their progeny is able to ingest food from conifers. We suggest that there is no change in host acceptance of nymphs between different instars, but nutritional needs could change between nymphal development stages. Therefore we investigated the emergence of adults starting from the earliest possible instar (2nd). Because the impact of low food quality or inhibitory components may accumulate and negative influence raise over time, 5th instar

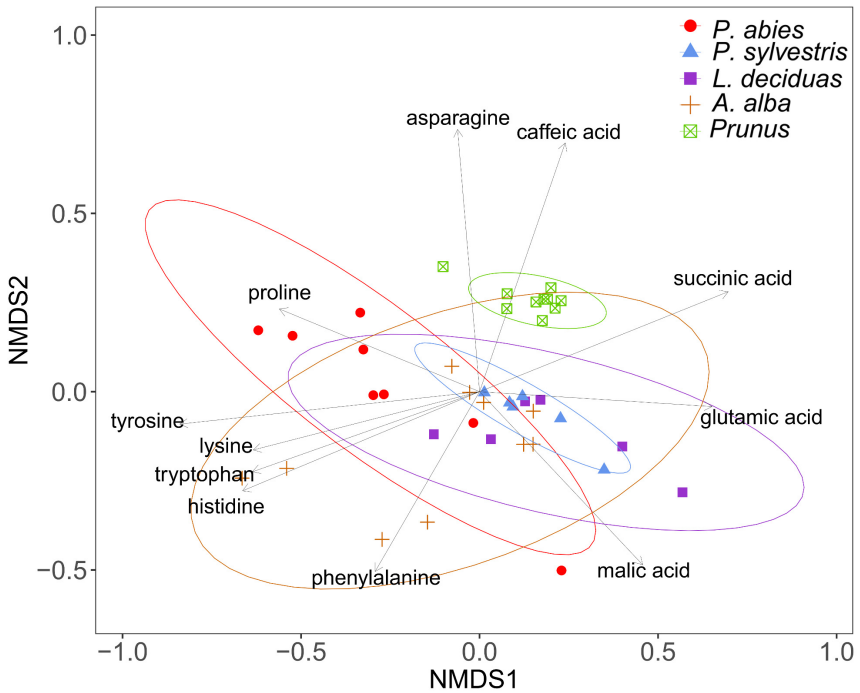

FIGURE 6 | Visualization of Bray–Curtis dissimilarities with NMDS plots (Stress: 0.13) of plant sap samples from spruce (n = 8), pine (n = 6), larch (n = 6), fir (n = 10), and *P. domestica* cv. Wavit (n = 10) derivatization with methyl chloroformate.

nymphs may able to compensate a short period on a non-optimal diet while early instars would suffer more from low food quality than later ones. But it is of crucial importance, whether *C. pruni* is able to fully develop from egg to adult stage on coniferous trees. Bioassays revealed that adult psyllids survived on coniferous trees, while nymphs did not develop and died, although they were able to ingest plant sap from conifer needles. Thus, the chemical composition of the respective conifer saps influences the nymphal survival and development. Therefore the plant saps of overwintering hosts were subsequently analyzed and compared to sap content of their reproduction host plant (*P. domestica*).

The GC-MS analysis revealed enormous differences in the chemical compositions of plant sap of the Rosacea species *P. domestica* cv. Wavit and the four studied conifer species. Especially the lack of sorbitol in all four conifers as well as the high amount of quinic acid and pinitol (which was not detected in *Prunus* trees) could be challenging for phloem feeding insects, which alternate between Rosacea and conifers during their life cycle. Even though it was known that spruce needles contain quinic acid, shikimic acid, fructose, glucose, sucrose, and pinitol (Schopf et al., 1982), to date it was unclear, in which proportions they occur in the phloem and xylem sap of coniferous trees, and how their proportions differ between tree species.

Until today it was a widespread belief that conifers are used by migrating *Cacopsylla* species like *C. pruni, C. picta*, and *C. melanoneura* for shelter during winter time, exclusively (Burckhardt et al., 2014; Jarausch and Jarausch, 2016). In the presented study we were able to show for the first time, that conifers are not only shelter plants for migrating species belonging to the genus *Cacopsylla*, but also an important food resource enabling their overwintering. Thus, the term "shelter plant" should hereafter be replaced by "overwintering host" or just "alternate host" plant.

Due to the lack of knowledge that psyllids feed on conifers, the effect of coniferous phloem constituents like quinic acid, shikimic acid, and pinitol on psyllid feeding behavior and development was not studied before. Pinitol is a cyclic polyol, which serves as osmoprotectant and is involved in a broad spectrum of physiological processes in plants (Chiera et al., 2006; Kordan et al., 2011; Saxena et al., 2013). It is found in conifers, legumes (Fabaceae) and Caryophyllales such as *Simmondsia chinensis* (Angyal and Macdonald, 1952; Dittrich and Korak, 1984; Guo and Oosterhuis, 1995; Chiera et al., 2006). D-pinitol induces oviposition of the Grass Yellow Butterfly *Eurema mandarina* (Mukae et al., 2016). However, an influence of pinitol from the phloem of alfalfa on phloem-feeding pea aphid could not be found (Campbell and Binder, 1984).

There is evidence, that psyllid adults and nymphs are tolerant to high osmotic pressures of their diets (Hall et al., 2010; Russell and Pelz-Stelinski, 2015). Therefore, we hypothesize no negative influence of pinitol on *C. pruni*, even if it occurs in high amounts in overwintering hosts. Quite the contrary, pinitol could act as mechanism of protection against freezing stress, as shown for other polyols (Bale, 2002). The freezing temperature of the green spruce aphid is reduced in the presence

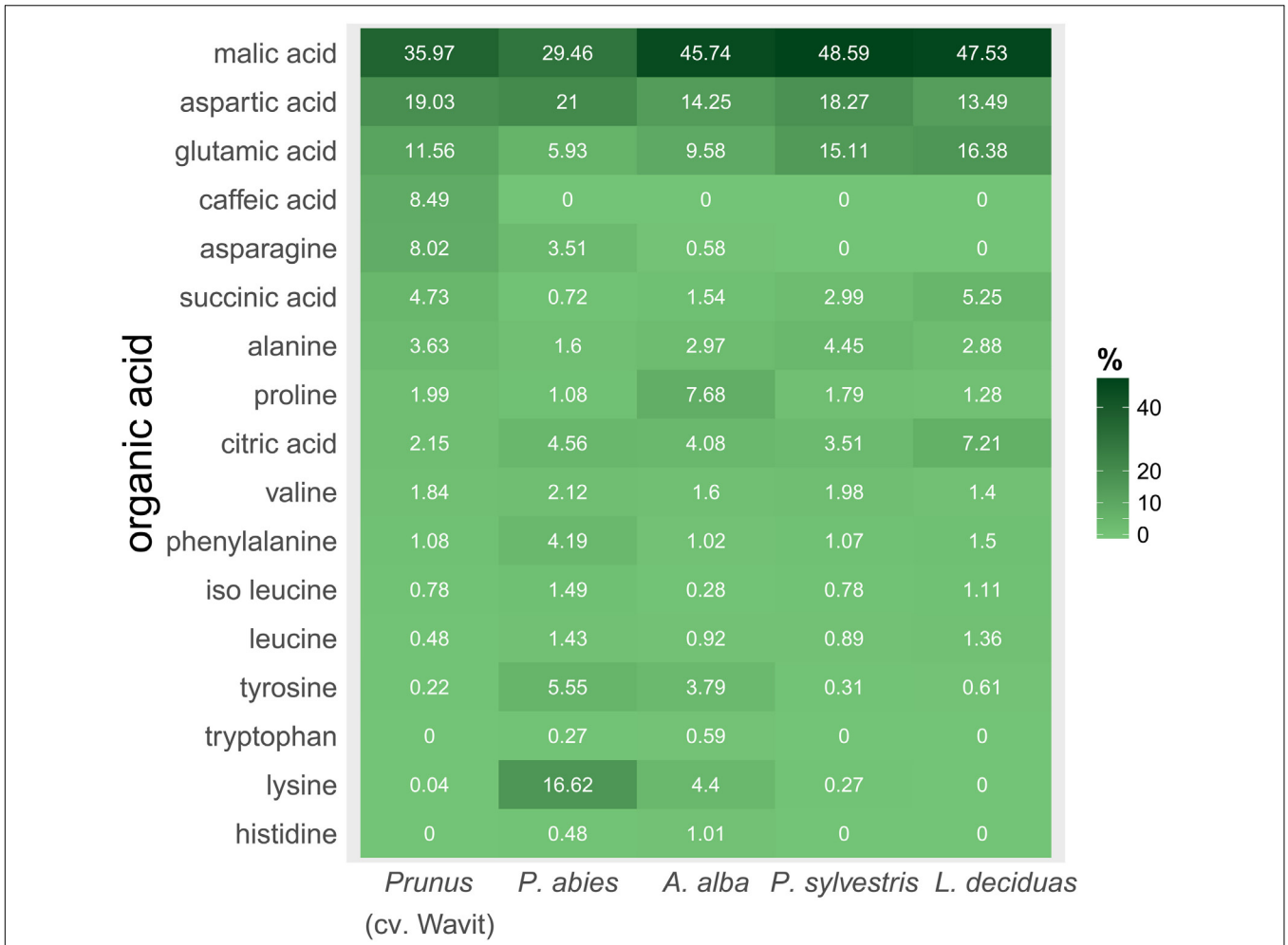

FIGURE 7 | Composition of organic acids in vascular bundle content of *P. domestica* cv. Wavit (n = 10), spruce (n = 8), fir (n = 10), pine (n = 6), and larch (n = 6). Plant sap was collected by centrifugation and derivatization with methyl chloroformate. Dark green indicates a high relative abundance of a respective organic acid, light green a low abundance. Numbers are mean values of relative abundance.

of mannitol in aphid hemolymph (Parry, 1979). Whiteflies accumulate sorbitol for thermo- and osmoprotection (Hendrix and Salvucci, 1998). Sømme (1965) found an accumulation of sorbitol in overwintering eggs of European red mite (*Panonychus ulmi*).

We found that sorbitol is the most abundant component in sap samples of *P. domestica* cv. Wavit, which is in accordance with the fact that sorbitol is most often found in Rosacea (Loescher, 1987). Sorbitol is also known to be accumulated in the phloem of apple trees (Bieleski, 1969) and is the most abundant soluble sugar in the phloem of pear and apple fruits (Zhang et al., 2004, 2014). Nevertheless, adult *C. pruni* can tolerate high amounts of sorbitol or pinitol in their diet. EPG recordings suggest that *C. pruni* (both adults and nymphs) also ingest xylem content (unpublished results), which could be a regulatory reaction to reduce the phloem's high osmotic pressure by dilution. Pompon et al. (2011) showed that aphids ingest more xylem sap after feeding on high concentrated sucrose diets to compensate osmotic unbalance. Moreover, for nymphal development the availability of amino acids (especially essential amino acids) could be of higher importance, as nitrogen content of food is an important limiting growth factor for phytophagous insects (Douglas, 2006). In accordance with Douglas (1993) we found asparagine besides aspartic acid and glutamic acid as one of the most abundant amino acids in young leaves of *Prunus*, while we found only low concentrations of glutamine in *Prunus* flush leaves. All plant species contained only low concentrations of the essential amino acids histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. To compensate for low quality of nitrogen in plant saps phloem feeders harbor microsymbionts (Douglas, 2006). Many psyllid species harbor the bacterial endosymbiont *Carsonella ruddii,* which provides its host with essential amino acids (Thao et al., 2000). Also representatives of the genus *Wolbachia*, *Arsenophonus* and other *Enterobacteriaceae* were found in psyllids (Baumann, 2005). Although the microsymbionts harbored by *C. pruni* are unidentified, differences in the symbiont community in adults

and nymphs were not expected, because vertical transmission of endosymbionts was shown for many species. Furthermore, recent studies indicated the transovarial transmission of *Arsenophonus* in *Cacopsylla pyricola* (Cooper et al., 2017).

We suggest that the inability of *C. pruni* nymphs to develop on coniferous trees is due to differences in organic acid availability. The caffeic acid, which is exclusively found in cv. Wavit, could play a key role in host acceptance of *C. pruni* and maybe act as a phagostimulant. Caffeic acid was found in several stone fruits like peaches and plums, which are typical host plants of *C. pruni* (Carbonaro et al., 2002; Lombardi-Boccia et al., 2004). However, not all of the components responsible for the separation of cv. Wavit from the coniferous species need to be of biological relevance. To unravel which components are actually important for proper development or which ones may inhibit nymphal growth, feeding experiments with nymphs on artificial diets are crucial. The analysis of excreted honeydew could suggest important information on how psyllids process plant nutrients. This study also revealed differences between the plant saps of the investigated coniferous trees. Therefore, a detailed analysis of EPG recordings from nymphs on the different tree species could be needful to identify feeding stimulants or deterrents and will be investigated in future. This knowledge could be used for development of an artificial diet system for rearing of *C. pruni* and screening for potential toxins against psyllids (Jancovich et al., 1997; Hall et al., 2010). Interestingly, although some of the migrating psyllids like *C. pruni* harbor phloem-limited plant pathogenic bacteria ('*Ca.* Phytoplasma' or '*Ca.* Liberibacter') and feed on conifers, the phytopathogens seem to be restricted to vector insects and their reproduction host plants (Gross, 2016). Because the genomes of Phytoplasma spp. lack metabolic genes but contain a lot of transporter systems, it is suggested that they depend strongly on the nutrition of their hosts (Oshima et al., 2004; Kube et al., 2008). Insight on the chemical composition of the phloem sap of host plants could support developing a culture media for phytoplasmas and may advance the research on phytoplasma diseases (Trivedi et al., 2016).

CONCLUSION

No mechanical nor chemical border prevents *C. pruni* adults and nymphs from feeding on conifers. Emigrants feed and survive on their overwintering hosts. Nymphs can feed on, but are not able to develop on spruce and fir. This is likely due to strong differences in the compositions of organic acids and sugars between plant saps of conifers and *P. domestica*. Furthermore, feeding experiments with nymphs on artificial diets should reveal which components are responsible for successful development of *C. pruni*. Additionally, more insight on phloem sap composition could open up new possibilities for phytoplasma cultivation and pathogen research.

AUTHOR CONTRIBUTIONS

JGa and JGr designed the study, contributed to the interpretation of the data, approved the final version of the manuscript, and ensured the accuracy and integrity of the work. JGa conducted the experiments and analysis and wrote the first draft of the manuscript, which was revisited and edited by JGr. JGr supervised the project.

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Publication 4

Phloem Metabolites of *Prunus* sp. rather than Infection with *Candidatus* Phytoplasma prunorum Influence Feeding Behavior of *Cacopsylla pruni* Nymphs

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Phloem Metabolites of *Prunus* Sp. Rather than Infection with *Candidatus* Phytoplasma Prunorum Influence Feeding Behavior of *Cacopsylla pruni* Nymphs

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Abstract
Phytoplasmas are specialized small bacteria restricted to the phloem tissue and spread by hemipterans feeding on plant sieve tube elements. As for many other plant pathogens, it is known that phytoplasmas alter the chemistry of their hosts. Most research on phytoplasma-plant interactions focused on the induction of plant volatiles and phytohormones. Little is known about the influence of phytoplasma infections on the nutritional composition of phloem and consequences on vector behavior and development. The plum psyllid *Cacopsylla pruni* transmits '*Candidatus* Phytoplasma prunorum', the causing agent of European Stone Fruit Yellows (ESFY). While several *Prunus* species are susceptible for psyllid feeding, they show different responses to the pathogen. We studied the possible modulation of plant-insect interactions by bacteria-induced changes in phloem sap chemistry. Therefore, we sampled phloem sap from phytoplasma-infected and non-infected *Prunus persica* and *Prunus insititia* plants, which differ in their susceptibility to ESFY and psyllid feeding. Furthermore, the feeding behavior and development of *C. pruni* nymphs was compared on infected and non-infected *P. persica* and *P. insititia* plants. Phytoplasma infection did not affect phloem consumption by *C. pruni* nymphs nor their development time. In contrast, the study revealed significant differences between *P. insititia* and *P. persica* in terms of both phloem chemistry and feeding behavior of *C. pruni* nymphs. Phloem feeding phases were four times longer on *P. insititia* than on *P. persica*, resulting in a decreased development time and higher mortality of vector insects on *P. persica* plants. These findings explain the low infestation rates of peach cultivars with plum psyllids commonly found in field surveys.

Keywords Plant-insect interaction · European stone fruit yellows · Vector development · Phytobiome · Phloem composition · Electropenetrography · Phytoplasma

Introduction

Phytoplasmas are phloem-restricted plant pathogenic bacteria, causing severe diseases in different plant species. Many of these phytoplasma-induced diseases affect agricultural crops (Bertaccini et al. 2014), resulting in high economic losses in crop production all over the world (Smith 1997). For example, the causal agent of the European stone fruit yellows (ESFY), *'Candidatus* Phytoplasma prunorum', infects different species of the genus *Prunus*. Infected trees suffer from severe symptoms, yield poorly, and exhibit dieback and decline (Kison and Seemüller 2001; Marcone et al. 1996; Nečas et al. 2017). Several *Prunus* species are susceptible to '*Ca*. P. prunorum' but vary in degree of symptom expression (Carraro et al. 2004a; Jarausch et al. 2000). Peaches, apricots and Japanese plums are severely affected (Kison and Seemüller, 2001; Torres et al. 2004), whereas *Prunus domestica, Prunus cerasifera* and *Prunus insititia* are found to be less affected (Kison and Seemüller 2001). Differences in response to ESFY infections also occur between cultivars within species (Koncz et al. 2017; Marcone et al. 1996; Richter 2002). Diverse symptoms are known to be associated with phytoplasma diseases. Besides structural changes of the vascular system, such as callose deposition, phloem necrosis, and hyperplasia

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(Musetti et al. 2016; Zimmermann et al. 2015), phytoplasma infections affect translocation of carbohydrates between source and sink plant organs and alter the metabolic compositions of leaf tissue (Christensen et al. 2005; Lepka et al. 1999; Prezelj et al. 2016). Because phytoplasmas are obligate parasites depending on their host plants and insects, they have small genomes that lack genes for some metabolic pathways and need to obtain nutrients from the host organism (Bai et al. 2006; Kube et al. 2008; Marcone et al. 1996; Marcone et al. 1999). While several studies highlighted changes in the chemistry of plant tissue due to phytoplasma infections, only few studies have determined the effects on the chemical composition of the phloem, which is the side of infection. In the most recent publication comparing the phloem composition of phytoplasma-infected vs. non-infected mulberry plants, Gai et al. (2014) found a change in the metabolic composition of phloem sap in response to phytoplasma infection. Their analysis revealed higher amounts of sucrose, abscisic acid (ABA), cytokinin and total content of free amino acids in phloem sap from infected than non-infected plants. In contrast, the phloem metabolome of coconut palms was not affected by lethal yellowing disease (Stemmer et al. 1982).

'*Ca*. P. prunorum' and other phytoplasma species of the 16SrX or apple proliferation group are transmitted by jumping plant lice or psyllids of the superfamily Psylloidea (Hemiptera: Sternorrhyncha) feeding on plant sieve tube elements (Weintraub and Beanland 2006). These psyllid-transmitted phytoplasmas as well as their vectors are closely related and associated with economically important diseases of fruit trees such as pear decline, apple proliferation and ESFY (Jarausch et al. 2019). The plum psyllid *Cacopsylla pruni* transmits '*Ca*. P. prunorum', the causal agent of ESFY by feeding on the phloem tissue of plants during reproduction (Carraro et al. 1998, 2004b). Little is known about the influence of phytoplasma infections on the nutritional composition of phloem and consequences on vector behavior and development. Amino acid composition, plant defense mechanisms and phytohormone concentrations (Dermastia 2019) could affect insect vector feeding on diseased plants. Although it is well known that nutritional quality and hormonal levels of plants in general impact insect performance and fitness (Cao et al. 2016; Pradit et al. 2019; Schoonhoven et al. 2010; Schweiger et al. 2014), much less is known about how plant infections with phloem-restricted bacteria impact insect fitness.

Cacopsylla picta emigrants that developed on *Malus domestica* trees infected with '*Ca*. P. mali' are smaller and their development is slightly elongated compared to psyllids that develop on healthy apple plants (Mayer et al. 2011). Consequently, females prefer healthy over infected plants for oviposition (Mayer et al. 2011). In contrast, the survival and reproduction of female *Macrosteles quadrilineatus* and *Dalbulus maidis* is enhanced on host plants infected with Aster Yellows-witches' broom phytoplasma (AY-WB) (Beanland et al. 2000; Purcell 1988; Sugio et al. 2011), while the infection of host plants with Bois Noir has no impact on growth of vector progeny (Kaul et al. 2009). By investigating the feeding behavior of Asian citrus psyllid (*Diaphorina citri*) with electropenetrography (EPG), Cen et al. (2012) recorded lower mean durations of phloem ingestion phase (E2) on plants inoculated with *'Candidatus* Liberibacter asiaticus' (CLas) than on uninfected plants. George et al. (2018) revealed lower total durations of E2 per psyllid in infected than uninfected *Citrus* plants. This reduction of phloem uptake is in accordance with the elongated developmental time of *D. citri* nymphs when reared on CLas-infected compared to uninfected *Citrus* plants (Pelz-Stelinski et al. 2010).

Prunus persica is highly susceptible to ESFY and shows severe symptoms and high mortality, while *P. insititia* is also susceptible but shows light symptoms and low mortality (Kison and Seemüller 2001). Therefore, we expected a significant influence of '*Ca*. P. prunorum' on the phloem metabolome of *P. persica.* A comparison with the metabolite composition of infected *P. insititia* could indicate whether phloem chemistry is influencing symptom manifestation or reveal components associated with phytoplasma tolerance. Killiny and Hijaz (2016) found higher abundance of amino acids involved in plant defense mechanisms in phloem sap of citrus varieties tolerant to CLas.

To investigate the interaction of '*Ca*. P. prunorum' with its natural plant environment, we analyzed sugars, sugar alcohols and organic acids in phloem centrifugates of infected and non-infected *Prunus* trees. Furthermore, we compared two *Prunus* species, which were differently affected by the infection (*P. persica* and *P. insititia*). To link the composition of primary plant metabolites of phloem centrifugates with vector development, we recorded and analyzed the feeding behavior and development of *C. pruni* nymphs on healthy and '*Ca*. P. prunorum'-infected plants. The importance of volatile organic compounds released by plants on *C. pruni* host preference and the importance of phloem chemistry on *C. pruni* development has been addressed previously (Gallinger et al. 2019, Gallinger and Gross 2018). Thus, the objective of the present research was to investigate the importance of gustatory cues on the host plant choice of *C. pruni* using two *Prunus* species that exhibit different degrees of sensitivity to ESFY phytoplasma infection.

Methods and Materials

Insects Overwintered *C. pruni* adults (remigrants) were collected by beating foliage above a collection tray in early spring (March and April). Psyllids were sampled at two different sites: the experimental field and surroundings of the Julius Kühn-Institut (JKI) in Dossenheim, Germany, and an

experimental *Prunus* orchard of Dienstleistungszentrum Ländlicher Raum Rheinpfalz (DLR), Neustadt an der Weinstrasse, Germany. Psyllids were reared on *Prunus spinosa* trees in insect cages (BugDorm, MegaView Science Co, Taiwan 47.5 × 47.5 × 93 cm), housed in a climate chamber at 20 °C (photophase) and 16 °C (scotophase) (L16:D8).

Plants Cultivars of *P. persica* (cv. South Haven) and *P. insititia* (cv. GF655–2) were used for experiments. *P. insititia* (cv. GF655–2) plants were dug out in October from the experimental field of the JKI and used for the experiments. Scion wood of *P. persica* cv. South Haven was grafted on one-year-old peach seedlings (cv. Montclar) as is common practice in fruit growing. All plants were grown in 1.8 L pots with clay substrate (Klasmann-Dielmann GmbH, Geeste, Germany). Plants were fertilized with ~ 500 ml Triabon (Compo Expert GmbH, Münster, Germany, 2 g/L) once in March and then weekly with 300–500 mL Wuxal (Hauert MANNA Düngerwerke GmbH, Nürnberg, Germany, 0.2%). *Prunus* trees were treated once with paraffin oil in March to prevent infestations with spider mites. All plants were housed in an insect free environment and treated weekly with nematodes *Steinernema feltiae* (SAUTTER & STEPPER GmbH, Ammerbuch, Germany) against fungus gnats. Polymerase chain reaction (PCR) analysis revealed naturally occurring phytoplasma infections in *P. insititia* plants from the field. Because we had no naturally infected *P. persica* plants, *P. persica* trees were graft-inoculated with *'Ca.* P. prunorum' ESFY Q06 from *Prunus marianna* GF 8–1 (*Prunus cerasifera x Prunus munsoniana*). Each tree was inoculated with two side-graftings of infected scion wood. Phytoplasma infestation was verified via PCR prior to experiments. Plants that were inoculated but infection with '*Ca.* P. prunorum' could not be verified were excluded from the experiments. Experiments were conducted between May and August in 2018 and 2019 during leaf and shoot development. No plants expressed inflorescences during the two years of experiments.

Development of C. pruni The influence of ESFY infection and host species on developmental time of *C. pruni* was investigated. Therefore, nymphs were placed on healthy and ESFY-infected *P. persica* cv. South Haven and *P. insititia* cv. GF 655–2 plants. Second instar nymphs were gently transferred with a fine brush from a *P. spinosa* plant to middle-aged fully expanded leaves from experimental plants. Ten nymphs were placed on each leaf and were caged with small gauze bags (10 × 12 cm). Due to logistic reasons, seven to ten bags (70–100 nymphs) were attached to plants from each species and ESFY infection status. Bags were monitored daily for nymph development and adult eclosion. Eclosed adults were counted daily and removed from the bags. The experiment continued for 49 days until all adults eclosed or nymphs died. The experiment was set up in May and ended in July 2019. Plants were inoculated with phytoplasmas two years before the experiment.

Electropenetrography (EPG) Fifth instar nymphs were collected from the rearing cages with *P. spinosa* plants one hour before EPG recordings (1 h starvation period). Nymphs were carefully cleaned with a wet cotton stick and were allowed to dry for about 10 min. A droplet of water-based silver glue (EPG-Systems, Wageningen, The Netherlands) was attached to the mesothorax of each nymph and a piece of fine gold wire (18 µm diameter, ca. 1 cm length) was fixed on the pronotum with a second droplet of silver glue. The gold wire was connected to a copper extension wire soldered to a brass insect pin. The pin was attached to the EPG probe. The reference electrodes were placed into the wet soil of the test plants. The feeding behavior of *C. pruni* nymphs was recorded with an 8-channel amplifier (model Giga-8d, EPG-Systems, Wageningen, The Netherlands) in a climate chamber at 23 °C with 60%–65% RH for 16 h (log-day period). Nymphs were placed on the adaxial surface of mature leaves (second to sixth fully expanded leaves). Plants and insects were housed in a grounded self-constructed Faraday cage made of zinc-coated bird cage wire (mesh size: 6.3 × 6.3 mm) during the recordings. Feeding patterns of 15 individuals were recorded from both ESFY-infected and non-infected *P. insititia* and *P. persica* plants. Only recordings from nymphs that showed 16 h of activity were included in the analysis, while nymphs that molted during the experiment were excluded. EPGs were recorded in May and June one and two years after inoculation with phytoplasmas (2018 and 2019). Data acquisition and analysis was performed with Stylet+ software (EPG-Systems, Wageningen, The Netherlands). Recordings were examined for occurrence of waveforms according to Bonani et al. (2010) and Civolani et al. (2011). Patterns corresponding to the start of penetration and the stylet position in the parenchyma (A, B, C1 and C2) were summarized as intracellular pathway phase (C). The phase between the parenchyma and the phloem was considered at phase D, which has been suggested as the transition phase between parenchyma and phloem. The two phloem feeding waveforms were E1 and E2, while the ingestion of xylem content was G. Finally, the non-probing (Np) phases were also annotated during which time insects were not penetrating the plant tissue with their stylets.

Collection of Sap Samples One phloem sap sample was collected from each tree with the centrifugation technique according to Hijaz and Killiny (2014). Briefly, the bark from young flush of *P. persica* and *P. insititia* plants was removed manually and sliced into 1–2 cm pieces with a clean scalpel. The bottom of a 0.5 ml Eppendorf tube was removed. Each tube was immersed in a second, larger tube (1.5 ml). To collect the phloem content, bark pieces were placed into the small tube

and centrifuged at 12.000 rpm at 4 °C for 10 min. The collected samples were stored at −80 °C until analysis. As we cannot totally exclude possible slight contamination from mesophyll cell content, we refer to the samples as phloem centrifugates henceforth. Phloem centrifugates were sampled in August 2018 one year after inoculation with phytoplasmas.

Measurement of °Brix Value To compare the absolute amount of soluble solid content in phloem centrifugates, °Brix values were measured with a handheld refractometer (type 45–81; Bellingham + Stanley Ltd., Tunbridge Wells, UK). The refractometer was calibrated with sucrose as standard. About 1 µl phloem centrifugate from either *P. insititia* ($n_{non\text{-}infected} = 6$, $n_{infected} = 6$) or *P. persica* ($n_{non\text{-}infected} = 11$, $n_{infected} = 7$) were used for measurements.

Derivatization of Phloem Centrifugates Silylation was used to analyze sugars, sugar derivates and organic acids in phloem centrifugates. Five µl of the samples were added to 60 µl of a 1.5 mmol ribitol internal standard solution (Sigma-Aldrich Chemie GmbH, Munich, Germany) and dried under nitrogen stream (Reacti-Vap, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Seventy µl of methoxyamine hydrochloride solution (MOX) in pyridine (2%) was added to each sample. Methoxyamine was allowed to react for 90 min at 37 °C stirring at adjustment of 7 (Reacti-Therm, Thermo Fisher Scientific Inc.). N-methyl—(N-trimethylsilyl) (MSTFA) was used as silylation reagent. After adding 90 µl MSTFA to each sample, the reaction was incubated for 60 min at 37 °C with stirring at adjustment of 7. The supernatant was transferred to a GC-MS vial with a glass insert. A second derivatization method using methyl chloroformate was used to optimize the detection of amino acids (Smart et al. 2010). Aliquots of 15 µl phloem centrifugates were mixed with 7.5 µl DL-norvaline (Sigma-Aldrich Chemie GmbH) as an internal standard (17 mmol in ultrapure water) and 180 µl sodium hydroxide (1 M). 167 µl methanol and 34 µl pyridine were added, followed by 20 µl MCF. Afterwards, the sample was vortexed for 30 s., and an additional 20 µl of MCF were added and the sample was mixed for 30 s. again. The alkylated derivatives were extracted by adding 150 µl chloroform and mixing for 10 s. After adding a 200 µl aliquot of sodium bicarbonate solution (50 mM), the samples were mixed again for 10 s. Silanized glass vials (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were used for the chemical reaction. The aqueous phase was discarded. To bind any remaining water, a few milligrams of anhydrous sodium sulfate were added to the organic layer. The supernatant was transferred to a GC-MS vial with a glass insert.

GC-MS Analysis Derivatized samples were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using a PerkinElmer Clarus R 680 GC system coupled to a Perkin Elmer quadrupole inert mass selective detector. For GC separation a nonpolar Elite-5MS (Crossbond 5% diphenyl −95% dimethyl polysiloxane, PerkinElmer) capillary column (30 × 0.25 mm id × 0.25 µm film thickness) was used. One µl of samples derivatized with MCF were injected with an open injector vent at 70 °C injector temperature, to purge out the solvent. After 0.5 min, the vent was closed and the injector temperature was raised to 290 °C after 1 min. Carrier gas flow rate (Helium, Air Liquide, Germany) was about 5 ml/min (column head pressure 130 kPa) and 30 ml/min split flow. The initial oven temperature of 80 °C was held for 2 min, followed by a temperature increase of 10 K/min up to 240 °C held for 3.5 min and a further increase to 300 °C at a rate of 20 K/min. The final temperature of 300 °C was held for 2 min. For the analysis of sap samples after silylation, 1.5 µl of each sample was injected with a split flow of 5 ml/min at 140 °C and the injector temperature was increased by 50 K/min to 250 °C. Column head pressure of Helium flow was set to 130 kPa. The GC temperature program was as follows: the initial oven temperature of 80 °C was held for 3 min, followed by an increase of 5 K/min up to 320 °C. The final temperature of 320 °C was held for 4 min. For all analysis the transfer line and ion source temperatures were set to 250 °C and 180 °C respectively. The quadrupole mass detector was operated in electron-impact (EI) mode at 70 eV. All data was obtained by collecting the full-scan mass spectra within the range of 35–550 m/z. Blank samples, reference standards and mixtures of alkanes (C8 - C20 and C 10- C40) were analyzed additionally according to both methods. Reference standards and suppliers are listed in the supplementary material (Table S1).

Identification and Quantification with AMDIS Chromatograms of sap sample derivates were analyzed using "Automated Mass spectral Deconvolution and Identification System" (AMDIS, V. 2.71; National Institute of Standards and Technology NIST, Boulder, CO). For the identification, the ion fragmentation patterns and retention indices of detected compounds were compared with standard compounds (Gross et al. 2019). Compounds that were not identified were annotated as unknowns. For quantification, the peak areas were integrated after deconvolution. Identification criteria were applied as follows: match factor had to be ≥ 80% and the relative retention index deviation ≤ 5% from reference value. The settings for deconvolution were: component width: 32; adjacent peak subtraction: one; resolution: medium; sensitivity: medium; shape requirements: low; level: very strong; maximum penalty: 20 and 'no RI in library': 20. Components with a signal to noise ratio < 50 were excluded from the analysis. Relative amounts of detected compounds after derivatization were calculated in relation to the respective internal standards norvaline and ribitol.

Statistical Analyses All statistical analyses were conducted in R version 3.5.3 (R Core Team 2017). Graphics were produced using the ggplot2 package (Wickham 2009). A parametric survival model (time-to-event analysis) was used to investigate the effect of plant species and the infection status of plants on the development of *C. pruni*. The model was fitted with an exponential distribution with the *survreg* function of the 'survival' package. Linear models (LMs) were used to determine the influence of the plant species and phytoplasma infection on the duration of waveforms per event (total), duration per nymph (mean) and the time to first occurrence of waveforms in EPG recordings from *C. pruni* nymphs. In case of non-normality of residuals, the data were transformed as specified in Table S2. The fit of models with the main effects '*Prunus* species' and 'ESFY infection status' and the interaction of these two factors was compared by second-order Akaike's information criterion (AICc) corrected for small samples. To analyze the occurrence (frequency) of individual waveforms per nymph, GLMs with quasi-Poisson distribution were used due to overdispersion. To compare models fitted with quasi-Poisson distribution the quasi-AICc (qAICc) was computed, using the model deviance instead of the likelihood and used in the *ICtab* function from 'bbmle' package (Bolker and R Development Core Team 2017). A LM was fitted with square root transformed °brix values, to analyze the influence of *Prunus* species and ESFY infection on the amount of total soluble solid content in phloem centrifugates. AICc was used to identify best model fit. Model assumptions were validated graphically as recommended by Zuur et al. (2009). The *emmeans* function from the 'emmeans' package (Lenth et al. 2019) was used to calculate the estimated marginal means and corresponding 95% confidence intervals and to determine differences between treatment levels. In case multiple pairwise comparison *p*-values were adjusted by the method of Tukey. Discrimination of the chemical composition of phloem centrifugates from infected and non-infected *Prunus* trees was calculated by a type II permutation multivariate analysis of variance (PERMANOVA) of the Bray-Curtis dissimilarities matrix. The PERMANOVA was calculated with the *adonis.II* function from 'RVAideMemoire' package (Hervé 2019). The dispersions of groups were tested for multivariate homogeneity (PERMDISP). Both analyses were calculated with $N = 10000$ permutations. The Bray–Curtis dissimilarities were visualized by non-metric multidimensional scaling (NMDS) plots. The scaling was standardized by Wisconsin double standardization and performed using the *metaMDS* function from 'vegan' package (Oksanen et al. 2019). Influence of main factors and interaction on the relative amount of total amino acids, sugars, sugar alcohols, and organic acids were analyzed by fitting linear models as described above. The model specifications were as reported in Table S3.

Results

Development of C. pruni After 49 days, all *C. pruni* nymphs had emerged to adults or died (Fig. 1). The development of *C. pruni* was significantly different between both *Prunus* species (*survreg*, $Z = 7.09$, $df = 1$, $P < 0.01$ $N = 370$). Fifty-seven and 60% of nymphs developed on healthy and phytoplasma-infected *P. insititia* plants, respectively; whereas, 15% of *C. pruni* emigrants emerged on healthy and 12% on diseased *P. persica* trees. Mean development time was 41 and 39 days on healthy and infected *P. insititia* plants, respectively. On average, *C. pruni* nymphs required 47 days for development on *P. persica* plants. Phytoplasma infection had no significant influence on the development of *C. pruni* nymphs (*survreg*, $Z = 0.34$, $df = 1$, $P = 0.73$, $N = 370$).

EPG Waveforms detected in EPG recordings from *C. pruni* nymphs were comparable to those specified for *C. pyri* (Civolani et al. 2011). The intracellular pathway phase (C), a phase that always occurred between parenchyma and phloem phases (D), two phloem patterns (E1 and E2), a xylem pattern (G) and non-probing phases (Np), as described by Civolani et al. (2011), were identified in the recordings.

Frequency The mean number of waveforms C, E2 and Np phases were neither affected by the *Prunus* species nor by infection status of the plants. Whereas the main effect of the plant species was significant for the occurrence of waveform D (*GLM*, $\chi^2 = 9.56$, $df = 1$, $P = 0.002$, $N = 60$) and E1 (*GLM*, $\chi^2 = 4.96$, $df = 1$, $P = 0.026$, N = 60), both waveforms were recorded more frequently from nymphs feeding on *P. persica* than on *P. insititia* plants (Table 1). The number of bouts of G was influenced by the infection status of the plants (*GLM*, $\chi^2 = 4.03$, $df = 1$, $P = 0.044$, $N = 60$). On average, nymphs accessed the xylem of healthy leaves 4 ± 3.27 and the xylem of infected leaves 2.7 ± 1.73 times during the 16 h recording period on both *Prunus* species (Table 1).

Mean Duration Per Psyllid Plant species had a strong effect on the mean duration of C (*GLM*, $F = 12.38$, $df = 1$, $P = <0.001$, $N = 60$), D (*GLM*, $F = 11.32$, $df = 1$, $P = 0.001$, N = 60), E1 (*GLM*, $F = 12.27$, $df = 1$, $P = <0.001$, N = 60), E2 (*GLM*, $F = 21.80$, $df = 1$, $P = <0.001$, N = 60) and Np (*GLM*, $F = 5.75$, $df = 1$, $P = <0.02$, N = 60) phases (Table 2). The mean duration per nymph in the pathway phase and phases (C) of non-probing (Np) were significantly longer during feeding on *P. persica* than on *P. insititia* (Table 2). Furthermore, the durations of D and E1 were longer on *P. persica* than *P. insititia* plants (Table 2). Nymphs feeding on *P. persica* plants spent about 75% of the time in non-ingestion phases, 13% ingesting phloem and 9% ingesting xylem. In contrast, nymphs feeding on *P. insititia* ingested phloem three times longer and the time

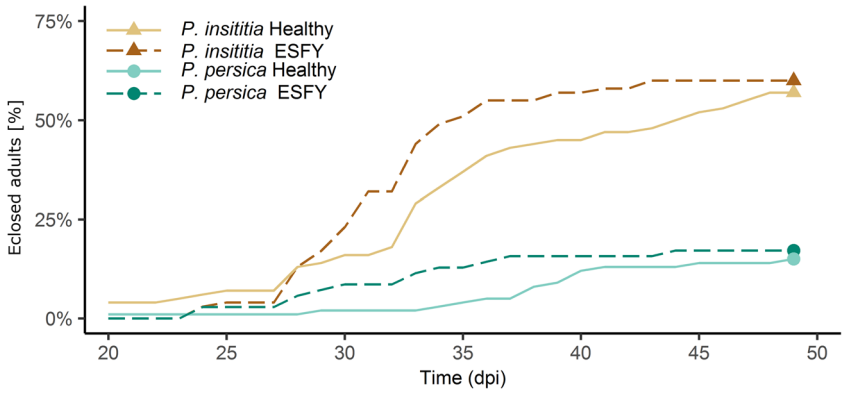

Fig. 1 Cumulative percentage of *C. pruni* nymphs completing development per day post infestation (dpi) on ESFY infected and healthy *P. insititia* ($n_{healthy} = 100$, $n_{ESFY} = 100$) and *P. persica* ($n_{healthy} = 100$, $n_{ESFY} = 70$) trees. Ten nymphs were caged together on each leaf

spent in non-feeding phases was 50% lower than on *P. persica* (Fig. 2b).

Mean Duration Per Event *Prunus* species and the interaction between species and ESFY infection status significantly affected the mean duration of waveforms not associated with phloem ingestion (C, D, E1 and Np) (Fig.3). C phases were shorter on infected than non-infected *P. persica* trees, whereas infection had no influence on the duration of C on *P. insititia* plants (Fig. 3). While the duration of E1 and Np phases was shorter on infected than non-infected *P. persica* plants, E1 and Np lasted longer in infected than in healthy *P. insititia* trees (Fig. 3). Phloem ingestion phases (E2) by nymphs feeding on *P. insititia* were significantly longer than those by nymphs feeding on *P. persica* (Table 3). On average, xylem phases lasted for 22.31 (± 2.13 SE) min. The mean duration per event was not affected by *Prunus* species nor ESFY infection (Table 3).

Time to First Occurrence The time until each waveform occurred the first time was not affected by plant species nor ESFY infection status of the plants (Table S4).

Chemistry of Phloem Centrifugates To estimate the total content of soluble sugar content, °Brix was measured from centrifugates. °Brix differed significantly as a function of *Prunus* species (*LM*, $F = 6.32$, $df = 1$; $P = 0.019$, $N = 30$). A higher °Brix value was measured in centrifugates from *P. insititia* plants (13.33 ± 1.76 SD) than from *P. persica* plants (11.33 ± 2.33 SD).

We found 10 amino acids and 9 organic acids (4 unidentified) in phloem centrifugates from *Prunus* trees after MCF derivatization (Table 4). The chemical composition of amino and organic acid in phloem centrifugates differed between the two *Prunus* species (Fig. 4a, *PERMANOVA*, $F = 3.97$, $df = 1$, $P = 0.009$, $N = 34$). The infection status as well as the interaction between infection and plant species

Table 1 Frequency of waveform events occurring in 16 h EPG recordings of *C. pruni* nymphs on *P. insititia* ($n_{healthy} = 15$, $n_{ESFY} = 15$) and *P. persica* ($n_{healthy} = 15$, $n_{ESFY} = 15$) trees

Waveform		*P. insititia*		*P. persica*		*P. insititia*	*P. persica*	model statistics*		
Frequency		mean ± SE	(min-max)	mean ± SE	(min-max)	emmean (lower-upper CI)	emmean (lower-upper CI)	influential factors	χ^2	P
C	healthy	35.87 ± 5.3	(5–73)	34.87 ± 3.06	(20–57)					
	ESFY	33.07 ± 4.69	(8–65)	39.27 ± 3.49	(20–58)					
D	healthy	7 ± 1.75	(1–29)	12.2 ± 1.27	(3–18)	6.77 (5.11–8.97)	11.70 (9.45–14.49)	species	9.560	0.002
	ESFY	6.53 ± 1.06	(1–14)	11.2 ± 2.15	(0–29)					
E1	healthy	11.07 ± 2.87	(1–48)	16.33 ± 1.82	(3–27)	10.8 (8.16–14.4)	16.3 (12.97–20.6	species	4.959	0.026
	ESFY	10.6 ± 1.8	(1–23)	16.33 ± 3.16	(0–34)					
E2	healthy	6.33 ± 1.8	(1–30)	8.07 ± 1.1	(1–16)					
	ESFY	6.53 ± 1.23	(1–17)	9.2 ± 2.2	(0–28)					
G	healthy	3.47 ± 0.75	(1–13)	4.53 ± 0.93	(1–15)	4.0 (3.13–5.11)				
	ESFY	2.2 ± 0.45	(0–6)	3.2 ± 0.42	(1–6)	2.7 (2.00–3.64)		infection	4.035	0.044
np	healthy	24.73 ± 3.66	(3–54)	17.47 ± 1.94	(4–32)					
	ESFY	23.07 ± 4.52	(3–62)	24.33 ± 3.11	(9–50)					

Mean (± SE) number per nymph, value range of occurrence and significant effects of *Prunus* species, ESFY infection of *Prunus* trees on the number of events. The estimated marginal means and the corresponding confidence intervals from the models are shown for significant factors

* Generalized linear models with quasi-Poisson distribution were used to analyze the effects of main factors and interactions on the frequency of waveforms events. Model statistics are presented for models simplified by removing nonsignificant factors due to AICc.

Table 2 Waveform durations per nymph from 16 h EPG recordings of *C. pruni* nymphs on *P. insititia* ($n_{healthy} = 15$, $n_{ESFY} = 15$) and *P. persica* ($n_{healthy} = 15$, $n_{ESFY} = 15$) trees

Waveform		*P. insititia*		*P. persica*		*P. insititia*	*P. persica*	model statistics*		
Duration / Nymph [min]		mean ± SE	(min-max)	mean ± SE	(min-max)	emmean (lower-upper CI)	emmean (lower-upper CI)	influencial factors	F	P
C	healthy	388.73 ± 56.44	(53.21–745.35)	584.07 ± 50.81	(200–858.99)	371 (309–439)	550 (474–632)	species	12.376	< .001
	ESFY	409.62 ± 47.86	(166.03–771.73)	542.98 ± 34.54	(317.31–767.75)					
D	healthy	6.33 ± 1.27	(1.36–18.6)	14.03 ± 1.79	(3.77–33.53)	5.49 (3.69–7.65)	11.21 (8.55–14.21)	species	11.321	0.001
	ESFY	6.11 ± 1.19	(1.27–19.1)	12.09 ± 2.99	(0–38.45)					
E1	healthy	4.17 ± 0.83	(0.91–11.85)	17.05 ± 4.56	(1.48–64.91)	4.77 (3.53–6.45)	10.04 (7.44–13.57)	species	12.27	< .001
	ESFY	5.53 ± 1.18	(0.22–17.26)	10.95 ± 2.29	(0–28.97)					
E2	healthy	386.22 ± 73.84	(3.79–798.54)	125.54 ± 38.62	(0.93–469.65)	382 (303.4–460)	124 (45.9–202)	species	21.798	< .001
	ESFY	376.83 ± 64.52	(12.17–741.01)	122.38 ± 38.7	(0–520.23)					
G	healthy	87.36 ± 28.1	(19.66–460.72)	88.89 ± 17.6	(21.02–233.72)	66.1 (46.9–93.0)				
	ESFY	50.71 ± 12.67	(0–172.48)	72.05 ± 16.83	(6.68–273.92)	40.2 (28.6–56.7)		infection	4.203	0.045
np	healthy	80.34 ± 12.97	(17.29–202.36)	119.34 ± 20.68	(39.84–290.29)	80.6 (56.8–108)	130.3 (99.5–165)	species	5.754	0.020
	ESFY	102.04 ± 20.75	(3.62–331.89)	180.12 ± 35.29	(35.67–496.26)					

Mean (± SE) duration, value range of occurrence and significant effects of *Prunus* species, ESFY infection of *Prunus* trees on the duration per nymph. The estimated marginal means and the corresponding confidence intervals from the models are shown for significant factors

*Linear models were used to analyze the effects of main factors and interactions on the frequency of waveforms events. Model statistics are presented for models simplified by removing nonsignificant factors due to AICc

had no significant effect on the discrimination between the phloem centrifugates (*PERMANOVA*, infection: $F = 1.85$, $df = 1$, $P = 0.117$, interaction: $F = 0.61$, $df = 1$, $P = 0.647$, N = 34). The variance in samples from *P. persica* was significantly higher than from *P. insititia* (*PERMDISP*, $F = 17.49$, $df = 1$, $P = 0.0002$, N = 34). Higher relative amounts of caffeic acid and one unidentified compound (unknown_RI206) were detected in phloem centrifugates from *P. insititia* plants compared to *P. persica* plants (Table 4). High relative amounts of asparagine, glutamic acid, citric acid and one unknown compound (unknown_RI2062) were found in phloem centrifugates from *P. persica* trees (Table 4). Overall, phloem centrifugates from *P. persica* plants contained higher relative amounts of amino acids than those from *P. insititia* (Fig. 5).

After TMS derivatization 5 organic acids, 7 sugars and sugar alcohols and 7 unidentified compounds were detected in phloem centrifugates (Table 4). The chemical composition of compounds after silylation differed significantly between the two *Prunus* species (Fig. 4b, *PERMANOVA*, $F = 23.33$, $df = 1$, $P = 9e\text{-}05$, $N = 40$); whereas, infection with '*Ca*. P. prunorum' had no influence on the composition of the detected metabolites (*PERMANOVA*, $F = 1.11$, $df = 1$, $P = 0.326$, N = 40). The variability between all four groups did not differ (*PERMDISP*, $F = 2.723$, $df = 3$, $P = 0.059$, N = 40). In general, *P. persica* samples showed a greater variance than samples from *P. insititia* (*PERMDISP*, $F = 4.891$, $df = 1$, $P = 0.033$, N = 40). Sorbitol was the most abundant compound in phloem centrifugates from both *Prunus* species (Table 4). Phloem centrifugates from *P. insititia* contained more sorbitol, sucrose and quinic acid than those from *P. persica* plants (Table 4). However, larger quantities of unknown_RI2519 were detected in samples from *P. persica* than form *P. insititia* (Table 4). The relative amount of sugars/sugar alcohols and organic acids was significantly higher in phloem centrifugates from *P. insititia* than from *P. insititia* plants (Fig. 5).

Discussion

It was shown previously that the plum psyllid, *C. pruni*, prefers *P. insititia* plants over *P. persica* plants in field (Gallinger et al. 2019). Our current results suggest that avoidance of *P. persica* appears to be beneficial to *C. pruni*, given that nymphs feeding on *P. persica* exhibited prolonged developmental time and reduced developmental success than observed on *P. insititia*. In contrast, nymphs seem not to be repelled by *P. persica* plants because they initiated stylet penetration behavior as fast as that observed on *P. insititia*. This is in accordance with recent findings from olfactometer assays, showing that *C. pruni* exhibit no preference between *P. insititia* and *P. persica* plants based on olfactory cues (Gallinger et al. 2019). Waveform D, as recorded by EPG, is

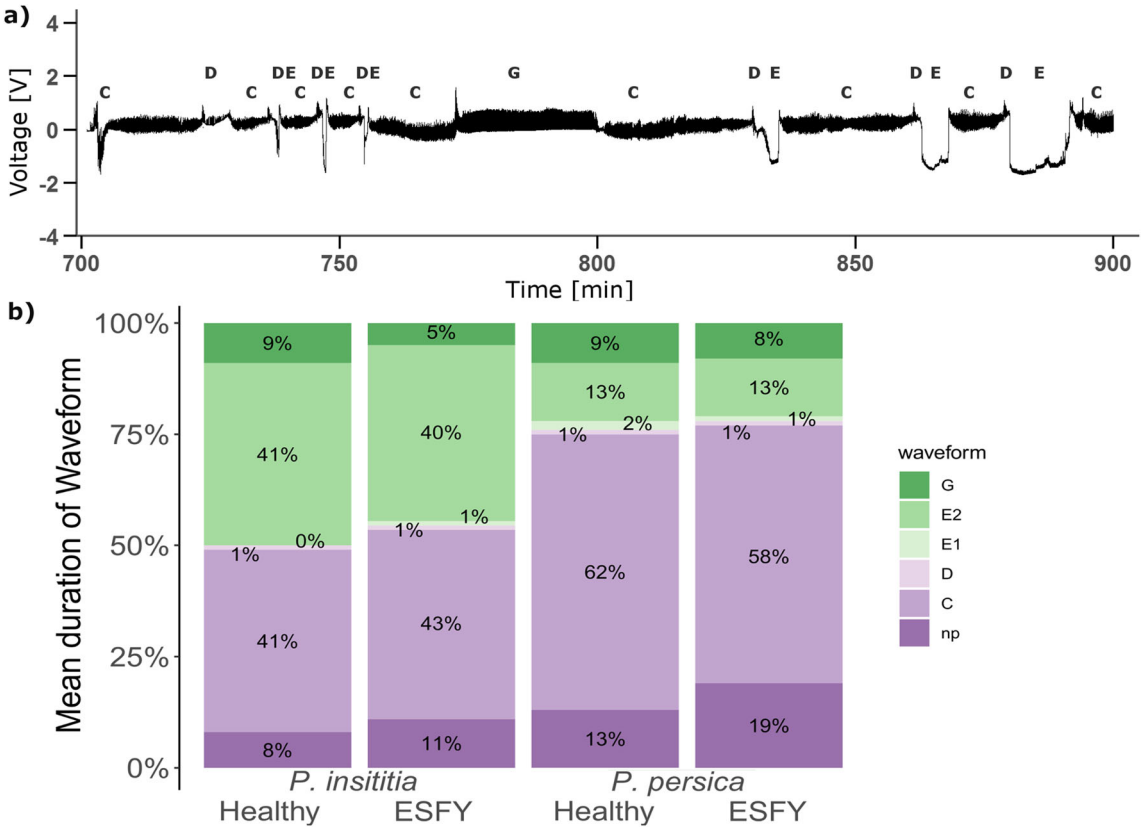

Fig. 2 a) Example of electropenetrography recording from *C. pruni* nymphs on a non-infected *P. persica* plant showing the classified waveforms: Intracellular pathway phase (C), transition phase between the parenchyma and the phloem (D), phloem salvation and ingestion (E), ingestion of xylem content (G) and the non-probing (Np) phases. b) Mean percentage duration of waveforms per psyllid detected during 16 h EPG recordings with *C. pruni* nymphs on *P. insititia* ($n_{healthy} = 15$, $n_{ESFY} = 15$) and *P. persica* ($n_{healthy} = 15$, $n_{ESFY} = 15$) trees. Additional explanations to particular waveforms are given in the text

thought to reveal the transition from parenchyma to phloem tissue feeding (Civolani et al. 2011). Extended periods in D phase could be a result of structural characteristics of the vascular tissue, but *C. pruni* nymphs were able to reach the phloem of *P. persica* as often and as fast as that of *P. insititia*. Thus, mechanical barriers like sclerenchymatous rings surrounding the phloem, which are shown to inhibit adult *D. citri* from reaching the vascular tissue (Ammar et al. 2014), are unlikely to be involved in this system. Regardless, the duration of phloem-feeding by *C. pruni* was drastically reduced on *P. persica* compared to *P. insititia* plants. Therefore, we suggest that the feeding preference for *P. insititia* may be rather influenced by phloem chemistry than by mechanical barriers.

Analyses of phloem centrifugates revealed significant differences between the chemical composition of *P. persica* and *P. insititia*. We recorded higher brix values for phloem centrifugates of *P. insititia* than those for *P. persica*. GC-MS analysis revealed higher amounts of sucrose, sorbitol and quinic acid in phloem of *P. insititia* compared to *P. persica*. Although phloem is generally rich in nutrients, amino acids essential for insects are rare and phloem-feeders have to face the challenge of overabundance of carbohydrates and high osmotic pressures comprising their diets (Douglas 2006; Douglas et al. 2006).

In contrast to differences in feeding behavior pattern between the two plant species, phytoplasma infections solely significantly decreased in both *Prunus* species the duration of xylem ingestion. The same effect of bacterial infection (CLas) of *Citrus* plants on feeding behavior of *D. citri* was found using EPG studies (Cen et al. 2012; George et al. 2018). Typically, psyllid nymphs exhibit reduced xylem ingestion and prolonged phloem ingestion compared to adults to meet their nutritional requirements (Civolani et al. 2011; George et al. 2018). Interestingly, in our study the reduction of xylem ingestion was not associated with prolonged phloem ingestion. It is assumed that xylem ingestion by phloem-feeders helps regulate fluid balance (Spiller et al. 1990). For example, potato aphids (*Macrosiphum euphorbiae*) use ingestion of xylem content to regulate their osmotic potential (Pompon et al. 2011). The higher amount of soluble carbohydrates in *P. insititia* did not lead to an increased ingestion of xylem content by *C. pruni* nymphs feeding on *P. insititia*. Nymphs spent more time feeding on phloem and their mortality was lower on *P. insititia* plants, which contained fewer amino acids

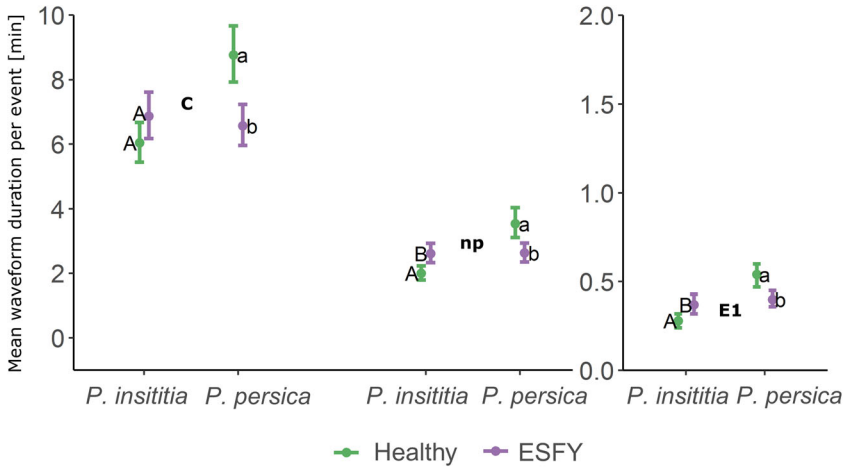

Fig. 3 Interaction plots of estimated marginal means and confidence intervals predicted from linear models of the mean duration per event of the waveform C, Np and E1 from EPG recordings of *C. pruni* nymphs feeding on healthy or ESFY infected *P. insititia* ($n_{healthy} = 15$, $n_{ESFY} = 15$) and *P. persica* ($n_{healthy} = 15$, $n_{ESFY} = 15$) trees

and higher amounts of sugars, than on *P. persica* plants, which had more amino acids and fewer sugars. The observed feeding behavior indicates that *C. pruni* is well adapted to *P. insititia* as a diet. Congruently, Jakobs and Müller (2018) documented that a high abundance of amino acids in phloem does not increase the developmental success of aphids in general. Instead, individual aphid species are adapted to specialized diet compositions (Jakobs et al. 2019). Interestingly, we found no increase in total sugar concentration (Brix value) in infected compared to non-infected plants. Thus, our results suggest that increased xylem phases are independent from phloem conditions and therefore might be based on differences in xylem metabolites.

Sugars can act as feeding stimulants for insects. The best-known example is sucrose, which stimulates feeding of many phytophagous insects, including aphids (Arn and Cleere 1971; Chapman 2003; Mittler and Dadd 1963). The sugar alcohol sorbitol is a characteristic phloem metabolite

Table 3 Duration of waveform per event from 16 h EPG recordings of *C. pruni* nymphs on *P. insititia* ($n_{healthy} = 15$, $n_{ESFY} = 15$) and *P. persica* ($n_{healthy} = 15$, $n_{ESFY} = 15$) trees

Waveform		*P. insititia*		*P. persica*		*P. insititia*	*P. persica*	model statistics*		
Duration / Event [min]		mean ± SE	(min-max)	mean ± SE	(min-max)	emmean (lower-upper CI)	emmean (lower-upper CI)	influential factors	F	P
C	healthy	10.84 ± 0.67	(0.05–174.54)	16.75 ± 1.23	(0.04–266.82)	6.04 (5.44–6.68)	8.76 (7.93–9.67)	species	9.549	0.002
	ESFY	12.39 ± 0.76	(0.12–130.96)	13.83 ± 1.23	(0.04–516.39)	6.87 (6.18–7.62)	6.57 (5.96–7.24)	infection	2.958	0.085
								interaction	16.448	< .001
D	healthy	0.9 ± 0.06	(0.06–4)	1.15 ± 0.05	(0.38–5.56)	0.77 (0.70–0.85)	1.05 (0.97–1.13)	species	20.818	< .001
	ESFY	0.94 ± 0.04	(0.14–2.42)	1.08 ± 0.05	(0.05–5.23)	0.85 (0.77–0.94)	0.94 (0.87–1.02)	infection	0.487	0.486
								interaction	5.315	0.022
E1	healthy	0.38 ± 0.03	(0.02–2.22)	1.04 ± 0.1	(0.05–11.89)	0.28 (0.24–0.32)	0.54 (0.47–0.60)	species	28.861	< .001
	ESFY	0.52 ± 0.05	(0.03–4.41)	0.67 ± 0.06	(0.03–7.97)	0.37 (0.32–0.43)	0.40 (0.36–0.45)	infection	0.814	0.367
								interaction	16.199	< .001
E2	healthy	60.98 ± 15.21	(0.23–768.65)	15.56 ± 4.95	(0.21–461.88)	9.17 (6.93–12.12)	3.28 (2.54–4.25)	species	42.507	< .001
	ESFY	57.68 ± 11.99	(0.09–706.66)	13.3 ± 2.72	(0.15–300.18)	12.53 (9.50–16.52)	4.49 (3.51–5.74)	infection	4.075	0.044
G	healthy	25.2 ± 6.06	(0.15–272.73)	19.61 ± 2.66	(0.17–142.97)					
	ESFY	23.05 ± 4.47	(0.71–135.65)	22.52 ± 3.68	(0.2–146.13)					
np	healthy	3.25 ± 0.35	(0.05–100.44)	6.83 ± 0.91	(0.1–201.55)	2.00 (1.79–2.23)	3.54 (3.11–4.04)	species	20.775	< .001
	ESFY	4.42 ± 0.33	(0.08–58.74)	7.4 ± 1.27	(0.04–282.72)	2.61 (2.33–2.93)	2.63 (2.35–2.94)	infection	0.017	0.8963
								interaction	22.568	< .001

Mean (± SE) duration, value range and significant effects of *Prunus* species, ESFY infection of *Prunus* trees and their interaction on the duration per event. The estimated marginal means and the corresponding confidence intervals from the models are shown for significant factors

* Linear models were used to analyze the effects of main factors and interactions on the frequency of waveforms events. Model statistics are presented for models simplified by removing nonsignificant factors due to AICc

Table 4 Mean relative amounts (± sd) of compounds detected via GC-MS analysis after derivatization

			P. insititia				*P. persica*			
	Compounds	**Retention Index**	**healthy**		**ESFY**		**healthy**		**ESFY**	
	TMS		**mean**	**± sd**	**mean**	**± sd**	**mean**	**± sd**	**mean**	**± sd**
Organic acids	Phosphoric acid	1271	0,000	0,000	0,019	0,014	0,053	0,049	0,125	0,155
	Malic acid	1489	0,083	0,048	0,121	0,053	0,123	0,083	0,151	0,089
	Citric acid	1816	0,502	0,292	0,377	0,278	0,753	0,975	0,561	0,710
	Quinic acid	1856	4,165	1,221	4,458	0,980	0,566	0,401	0,407	0,191
	Galactaric acid	2001	0,002	0,005	0,005	0,006	0,000	0,000	0,000	0,000
Sugars and sugaralcohols	Xylose	1645 / 1656	0,000	0,000	0,001	0,003	0,010	0,014	0,002	0,005
	Fructose	1869 / 1885	0,275	0,058	0,459	0,198	0,428	0,280	0,533	0,293
	Glucose	1895 / 1912	0,520	0,223	0,618	0,171	0,863	0,627	0,707	0,281
	Mannitol	1926	0,022	0,003	0,023	0,014	0,179	0,641	0,961	2,848
	Sorbitol	1933	16,978	3,238	15,017	3,824	11,293	3,287	10,325	2,066
	Myo-inositol	2086	0,197	0,029	0,325	0,105	0,188	0,141	0,201	0,188
	Sucrose	2629	6,439	2,414	5,122	2,768	3,187	1,573	3,209	1,846
Unidentified	unknown_RI1526	1526	0,040	0,014	0,042	0,010	0,030	0,020	0,047	0,026
	unknown_RI1997	1997	0,046	0,005	0,055	0,014	0,059	0,044	0,047	0,040
	unknown_RI2519	2519	0,879	0,264	0,698	0,315	4,376	2,548	4,091	2,843
	unknown_RI2860	2860	0,042	0,024	0,037	0,036	0,001	0,003	0,009	0,009
	unknown_RI2886	2886	0,213	0,115	0,256	0,105	0,078	0,079	0,102	0,173
	unknown_RI3116	3116	0,033	0,014	0,061	0,056	0,027	0,033	0,030	0,037
	unknown_RI3389	3389	0,054	0,027	0,111	0,067	0,052	0,054	0,167	0,176
	MCF									
Amino acids	Asparagine	1385	0,067	0,093	0,328	0,406	3,882	4,489	4,085	6,291
	Proline	1386	0,017	0,019	0,038	0,069	0,126	0,286	0,231	0,575
	Aspartic acid	1454	0,562	0,268	0,950	0,353	0,903	0,826	1,315	1,248
	Serine	1521	0,000	0,000	0,004	0,012	0,000	0,000	0,010	0,020
	Glutamic acid	1580	0,722	0,389	0,897	0,279	1,511	1,062	1,387	0,813
	Phenylalanine	1720	0,037	0,016	0,057	0,041	0,037	0,030	0,051	0,044
	Lysine	2012	0,021	0,026	0,014	0,013	0,048	0,067	0,050	0,096
	Histidine	2067	0,003	0,007	0,015	0,018	0,071	0,074	0,193	0,371
	Tyrosine	2186	0,020	0,014	0,038	0,033	0,018	0,018	0,034	0,030
	Tryptophan	2377	0,023	0,024	0,084	0,150	0,030	0,053	0,010	0,018
organic acids	Malic acid	1107	1,198	0,468	1,376	0,434	0,955	0,510	0,826	0,387
	Cinnamic acid	1373	0,016	0,014	0,010	0,013	0,000	0,000	0,000	0,000
	Citric acid	1460	4,823	3,032	3,645	2,857	8,642	9,980	2,281	1,699
	Salicylic acid	1526	0,006	0,009	0,008	0,009	0,002	0,007	0,011	0,018
	Caffeeic acid	2232	0,904	0,649	1,254	0,905	0,191	0,251	0,211	0,164
	unknown_RI1602	1602	0,178	0,165	0,150	0,156	0,444	0,499	0,077	0,115
	unknown_RI1654	1654	0,479	0,340	0,404	0,329	1,286	1,476	0,312	0,256
	unknown_RI1879	1879	1,797	0,767	1,484	1,198	2,860	1,282	2,077	1,570
	unknown_RI2062	2062	0,426	0,227	0,336	0,358	0,005	0,007	0,006	0,007

Min Max

Amounts of organic acids, sugars, sugaralcohols and unknown compounds after silylation of phloem centrifugates from healthy or ESFY infected *P. insititia* ($n_{healthy} = 6$, $n_{ESFY} = 10$) and *P. persica* ($n_{healthy} = 14$, $n_{ESFY} = 10$) trees are relative to internal standard ribitol. Amounts of amino acids and organic acids after MCF derivatization of phloem centrifugates from healthy or ESFY infected *P. insititia* ($n_{healthy} = 5$, $n_{ESFY} = 12$) and *P. persica* ($n_{healthy} = 10$, $n_{ESFY} = 7$) trees are relative to the internal standard norvaline. Colors range from green (min) to red (max) (see below)

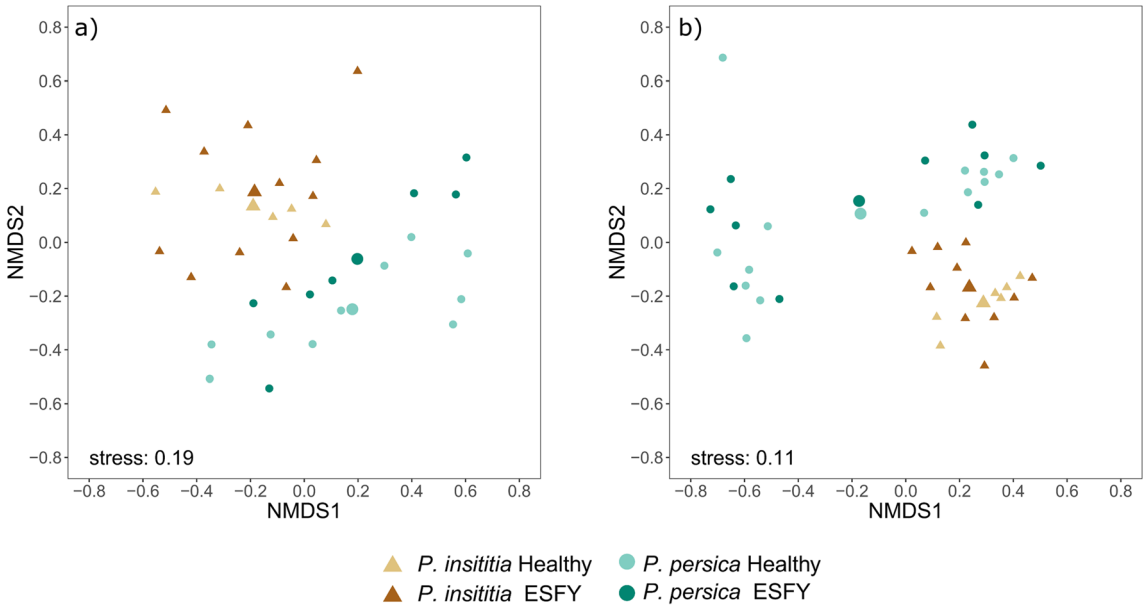

Fig. 4 Visualization of Bray–Curtis dissimilarities with non-metric multidimensional scaling (NMDS) plots of phloem centrifugates from ESFY-infected (dark) and non-infected (light) *Prunus* trees. a) amino and other organic acids from *P. insititia* (brown triangles, $n_{healthy} = 5$, $n_{ESFY} = 12$) and *P. persica* (green dots, $n_{healthy} = 10$, $n_{ESFY} = 7$) trees and b) sugars and organic acids from *P. insititia* ($n_{healthy} = 6$ $n_{ESFY} = 10$) and *P. persica* ($n_{healthy} = 14$, $n_{ESFY} = 10$) trees. Large triangles and circles visualize group centroids

of plants belonging to the Rosaceae and could therefore play a central role in host acceptance of psyllid species feeding on *Prunus* spp., *Malus* spp. or *Pyrus* spp. (Spiraeoideae: Rosaceae). The chemosensory sensilla from the mouthparts of *C. pruni* have not been described, but phagostimulatory cells that respond to sorbitol are known to occur in caterpillars specialized on rosaceaes plant species (Chapman 2003). Although sugars stimulate feeding by herbivores, phloem-feeders must excrete surplus non-assimilated sugars as honeydew (Ammar et al. 2013; Douglas 2006; Le Goff et al. 2019). Thus, future analysis of honeydew from nymphs could reveal components essential for proper development of *C. pruni* (Le Goff et al. 2019).

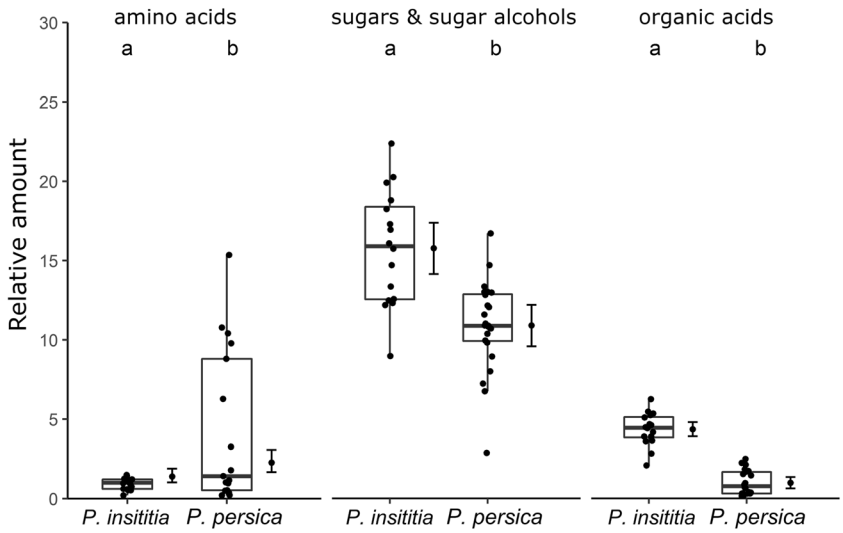

Fig. 5 Mean relative amount of total amino acids of phloem centrifugates from *P. insititia* ($n = 17$) and *P. persica* (n = 17) after MCF derivatization, sugars and organic acids in phloem centrifugates from *P. insititia* ($n = 16$) and *P. persica* ($n = 24$) after silylation. Amino acids (asparagine, proline, aspartic acid, serine, glutamic acid, phenylalanine, lysine, histidine, tyrosine and tryptophan) have been quantified relative to the internal standard norvaline. Organic acids (phosphoric acid, malic acid, citric acid, quinic acid and galactaric acid), sugars and sugaralcohols (xylose, fructose, glucose, sucrose, mannitol, sorbitol and myo-inositol) after silylation have been quantified relative to internal standard ribitol. Boxes correspond to the 25th and 75th percentiles, medians are shown as lines, and whiskers extend to 1.5 times of the interquartile ranges. Dots represents raw values. Corresponding means and confidence intervals predicted for significant factors from linear models are shown on the right of each box

Of the compounds detected in the phloem centrifugates (Table 4), caffeic acid is of particular interest. Its possible positive influence on the feeding behavior of *C. pruni* deserves further investigation, because this metabolite was also detected in phloem sap of *Prunus domestica* but not in conifers, which are no suitable hosts for feeding and development of *C. pruni's* offspring (Gallinger and Gross 2018). Hydroxycinnamic acids are commonly known as constitutive plant defenses against herbivores (Rehman et al. 2012). For example, chlorogenic acid is related to thrips resistance in plants (Leiss et al. 2009; Leiss et al. 2013). To our knowledge, the influence of phenolics on feeding behavior of psyllids has not been studied. Among psyllid species, phagostimulants have only been investigated for *D. citri* and appear to result from degradation products of common citrus volatiles (George et al. 2016; Lapointe et al. 2016). Therefore, further experiments should investigate whether reduced feeding of *C. pruni* nymphs is based on feeding deterrents or the lack of important metabolites that stimulate feeding on *P. persica.*

In the current study, we investigated the impact of phytoplasma infection on the phloem chemistry of its host plant. For this purpose, we compared two different plant-phytoplasma combinations: a less susceptible *Prunus* species naturally infected by a *'Ca*. P. prunorum' strain, which induced no symptoms, and a highly susceptible *Prunus* species inoculated with a '*Ca*. P. prunorum' isolate that induces characteristic symptoms in the susceptible species. Neither type of infection with either '*Ca*. P. prunorum' strain caused major changes in the composition of detected sugars, sugar alcohols and organic acids in *P. persica* or *P. insititia* plants. Naturally infested *P. insititia* plants were possibly colonized by a different phytoplasma strain than graft-inoculated *P. persica* plants. The virulence of phytoplasmas mainly depends on the combination of scions and isolates, but is also influenced by the rootstock. Kison and Seemüller (2001) investigated the virulence of different *'Ca*. P. prunorum' strains in combination with different *Prunus* species. *P. persica* scions and rootstocks suffered from infections with all tested ESFY isolates but to varying degrees. In contrast, *P. insititia* rootstocks have been less susceptible to all tested ESFY isolates. Regarding the current results, we cannot exclude the possibility that other combinations of scions, rootstocks and phytoplasma strains could affect changes to phloem composition. The feeding behavior of *C. pruni* nymphs was partly influenced by phytoplasma colonizing host plants. The interaction of the main factors (*Prunus* species and ESFY infection) affected feeding behavior. This supports the hypothesis that ESFY infections differentially affect *P. persica* and *P. insititia* trees. Shortened intracellular pathway phases (C) could indicate that *C. pruni* nymphs were able to reach the sieve-tube elements faster on infected *P. persica* than on uninfected plants. This might be a consequence of structural changes in phloem tissue, as enlargement of whole midribs is a characteristic symptom of ESFY in *P. persica* plants (Marcone et al. 1996).

Indeed, investigations have reported that *C. pruni* can survive and reproduce on *P. persica* in general (Carraro et al. 2004a; Fialová et al. 2004). However, we are the first to show that *P. persica* (peach) is a less suitable host for plum psyllids, which is clearly demonstrated by the low number of nymphs that developed successfully on *P. persica* plants. This is in accordance with findings from field surveys of *C. pruni* feeding on different *Prunus* species (Carraro et al. 2002; Gallinger et al. 2019; Mergenthaler et al. 2017). The measurement of abundance of *C. pruni* was monitored in these field surveys under the same conditions as in current study: non-grafted *P. insititia* rootstocks were compared with grafted *P. persica* scions on other rootstocks as this is common agricultural practice in fruit growing. To our knowledge there are no studies describing the influence of grafting on phloem chemistry of *Prunus* species, but it has been shown that rootstock species influences plant growth and fruit quality (Melnyk 2017). The rootstock-scion interaction can also influence psyllid feeding behavior, as grafting on resistant interstocks reduced scion susceptibility to pear psylla, *Cacopsylla bidens* (Shaltiel-Harpaz et al. 2018).

Even though *P. persica* is not a preferred host plant of *C. pruni,* trees are highly susceptible to phytoplasma infections and suffer from severe symptoms. Manifestation of symptoms could be elicited by physical changes of the vascular system and secondary metabolites, as an infection with *Ca*. P. prunorum' induces the release of phytohormones and the deposition of callose in *P. persica* plants (unpublished data). Phytohormones could affect the feeding behavior of vector nymphs on ESFY-infected *P. persica* trees. There is evidence that plant defense mechanisms mediated by phytohormones are induced in response to *'Ca*. P. prunorum' infestations in apricot trees, which may lead to recovery from and tolerance to ESFY (Osler et al. 2014; Osler et al. 2016). Microbial phytopathogens induce hormonal changes in plants both directly and indirectly and this has been demonstrated for bacteria, fungi and viruses (Dermastia 2019; Killiny 2017; Ma and Ma 2016; Mauck et al. 2016). In many pathosystems these modifications are proven to alter the behavior of vector insect either directly or indirectly via volatile organic compounds (Bak et al. 2019; Martini et al. 2017; Martini et al. 2018; Mayer et al. 2008a, 2008b; Rid et al. 2016). Further, the infection status of the vector itself influenced the behavior (Mayer et al. 2008b). In this regard, the feeding and oviposition preferences of adult *C. pruni*, as influenced by their infection status, should be investigated to evaluate the possible effect on the transmission and spread of bacteria. Even though psyllid nymphs are less mobile than winged adults, nymphs spend more time feeding on phloem tissue (E1 and E2) (Civolani et al. 2011; George et al. 2018). As a result, acquisition of bacteria is higher when adults emerge from nymphs

that fed on infected plants than when uninfected adults feed on infected plants (George et al. 2018; Inoue et al. 2009; Pelz-Stelinski et al. 2010). Consequently, transmission efficiency is higher when bacteria are acquired during the nymph than adult stage (Pelz-Stelinski et al. 2010). Since we found no negative effect of host plant phytoplasma colonization on development of *C. pruni* nymphs, it is possible that emerged adults contained high titers of bacteria and were capable of efficient pathogen inoculation.

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Author Contributions J Gallinger and J Gross conceived and designed the experiments. J Gallinger conducted the experiments, analyzed the data and wrote the first draft of the manuscript, which was revisited and edited by J Gross. J Gross supervised the project.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Table S1: Suppliers of reference standards used for GC-MS analysis.

Standard	Supplier
Alanine	Sigma-Aldrich Chemie GmbH (Munich, Germany)
Aspartic acid	
Cysteine	
Glutaminic acid	
Histidine	
Iso-leucine	
Leucine	
Lysine	
Myo-isonitol	
Pinitol	
Proline	
Ribitol	
Salicylic acid	
Threonine	
Tryptophan	
Valine	
Xylose	
Arginine	SERVA Electrophoresis GmbH (Heidelberg, Germany)
Phenylalanine	
Glycine	Carl Roth GmbH + Co. KG (Karlsruhe, Germany)
Methionine	
Serin	
Malic acid	
Succinic acid	
Arabinose	
Sucrose	
Asparagine	Merck KGaA (Darmstadt, Germany)
Mannitol	
Glucose	
Galactose	
Sorbitol	AppliChem GmbH (Darmstadt, Germany)
Glutamine	
Citric acid	Acros Organics (Thermo Fisher Scientific, Geel, Belgium)

Table S2: Specification of linear models analyzing waveform parameters form EPG recordings of *C. pruni* nymphs (L5) feeding on healthy and ESFY infected *P. insititia* und *P. persica* leaves.

waveform mean duration / nymph	Data transformation	waveform mean duration / event	Data transformation
C	sqrt	C	log+1
D	sqrt	D	log+0.01
E1	log+1	E1	log+0.01
E2	none	E2	log+0.01
G	log+1	G	log+10
np	sqrt	np	log+0.01

Table S3: Specification and results of linear models analyzing the relative amount of total amino acids, sugars and organic acids in phloem sap samples from healthy and ESFY infected *P. insititia* und *P. persica.*

Relative amount of	Data transformation	Factor	F-value	Pr(>F)
Amino acids	log+0.01	species	4.376	0.044
Sugars and sugaralcohols	none	species	22.536	<0.001
Organic acids	none	species	140.29	<0.001

Table S4: Specification and results of linear models analyzing the time (min) until first occurrence of each waveform in EPG recordings from *C. pruni* nymphs (L5) feeding on healthy and ESFY infected *P. insititia* und *P. persica* leaves.

Time to first occurrence of waveform	Data transformation	Factor	F-value Full model	Pr(>F) Full model	Best model AICc
C	log+0.01	species	0.166	0.685	null model
		infection	0.064	0.801	
		interaction	0.899	0.347	
D	sqrt	species	1.712	0.196	null model
		infection	0.025	0.874	
		interaction	0.196	0.660	
E1	sqrt	species	1.714	0.196	null model
		infection	0.024	0.878	
		interaction	0.198	0.658	
E2	sqrt	species	2.129	0.151	null model
		infection	1.722	0.195	
		interaction	0.906	0.345	
G	log+1	species	0.028	0.869	null model
		infection	0.164	0.687	
		interaction	0.106	0.746	
np	log+1	species	0.463	0.499	null model
		infection	0.293	0.590	
		interaction	0.027	0.870	

waveform	**P. insititia**				**P. persica**			
Time to first occurrence of waveform	**healthy**		**ESFY**		**healthy**		**ESFY**	
	mean ± SE	**(min-max)**	**mean ± SE**	**(min-max)**	**mean ± SE**	**(min-max)**	**mean ± SE**	**(min-max)**
C	6.86 ± 3.03	(0.64 - 37.61)	9.14 ± 5.13	(1.32 - 80.41)	11.08 ± 6.41	(1.2 - 99.49)	19.42 ± 15.43	(0.64 - 235.09)
D	262.8 ± 56.83	(50.25 - 897.45)	280.8 ± 60.64	(37.35 - 910.98)	200.46 ± 30.52	(75.72 - 512.48)	232.83 ± 68.13	(0 - 823.61)
E1	263.86 ± 56.93	(50.78 - 899.55)	282.13 ± 60.69	(38.02 - 912.76)	201.52 ± 30.52	(77.08 - 513.64)	233.63 ± 68.12	(0 - 824.38)
E2	309.67 ± 57.56	(50.93 - 900.46)	293.74 ± 61.3	(38.1 - 912.98)	273.85 ± 47.62	(77.29 - 684.07)	210.24 ± 65.36	(0 - 824.83)
G	113.43 ± 27.21	(12.12 - 437.6)	196.46 ± 60.62	(0 - 930.62)	103.31 ± 23.24	(12.08 - 275.94)	196.26 ± 74	(10.55 - 953.32)
np	29.93 ± 10.77	(1.17 - 159.48)	27.98 ± 9.36	(3.99 - 135.78)	28.44 ± 9.04	(2.28 - 116.64)	43.21 ± 18.57	(2.62 - 287.6)

6. Appendix

Ehrenwörtliche Erklärung

Ich erkläre hiermit ehrenwörtlich, dass ich die vorliegende Arbeit entsprechend den Regeln guter wissenschaftlicher Praxis selbstständig und ohne unzulässige Hilfe Dritter angefertigt habe. Sämtliche aus fremden Quellen direkt oder indirekt übernommenen Gedanken sowie sämtliche von Anderen direkt oder indirekt übernommenen Daten, Techniken und Materialien sind als solche kenntlich gemacht. Die Arbeit wurde bisher bei keiner anderen Hochschule zu Prüfungszwecken eingereicht.

Dossenheim, den _____

_____

(Jannicke Gallinger)

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Curriculum Vitae

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Professional experience

Since 03/2015	PhD student at the Julius Kuehn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture Project: "PRUNI-REPEL: Establishment of a Repellent Release System for the Control of Plum Leaf Sucker *Cacopsylla pruni* as a Part of a Push-and-Pull Strategy"
2012 – 2014	Bielefeld University, Germany Graduate assistant in the Chemical Ecology working group
08/2010 – 10/2010	Intern at Museum am Schölerberg, Osnabrück (museum of natural history)

Education

10/2010 – 09/2014	M.Sc. Ecology and Diversity Bielefeld University, Germany Thesis: "Different arbuscular mycorrhizal fungal species affecting *Plantago lanceolata* with focus on plant defense mechanisms"
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Publications – peer-reviewed

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